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(54) Title: CHITIN SYNTHASE 1

(57) Abstract

A polynucleotide encoding chitin synthase (CHS1), an enzyme essential for cell wall synthesis and yeast cell growth, is provided. A maltose responsive promoter (MRP) isolated using the promoter library of the invention is also described. The present invention also provides a vector for isolation of a eukaryotic regulatory polynucleotide, i.e., promoter. The vector is useful in the method of the invention which comprises identifying a eukaryotic regulatory polynucleotide, i.e., promoter region, by complementing the growth of an auxotrophic host cell containing the vector of the invention, which includes a promoter region operably linked to a promoterless auxotrophic gene. The vector is introduced into the host cell chromosome by targeted integration. Also provided is a library containing host cells having the vector of the invention integrated in the chromosome of the host cell.

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TITLE OF THE INVENTION

CHITIN SYNTHASE 1

Field of the Invention

This invention relates generally to the field of gene expression and specifically to genes essential for growth and to a vector and a method for the identification of such genes, as well as identification of eukaryotic promoters.

Background of the Invention

Many eukaryotic genes are regulated in an inducible, cell type-specific or constitutive manner. There are several types of structural elements which are involved in the regulation of gene expression. There are *cis*-acting elements, located in the proximity of, or within, genes which serve to bind sequence-specific DNA binding proteins, as well as *trans*-acting factors. The binding of proteins to DNA is responsible for the initiation, maintenance, or down-regulation of transcription of genes.

The *cis*-acting elements which control genes are called promoters, enhancers or silencers. Promoters are positioned next to the start site of transcription and function in an orientation-dependent manner, while enhancer and silencer elements, which modulate the activity of promoters, are flexible with respect to their orientation and distance from the start site of transcription.

For many years, various drugs have been tested for their ability to alter the expression of genes or the translation of their messages into protein products. One problem with existing drug therapy is that it tends to act indiscriminately on genes and promoters and therefore affects healthy cells as well as neoplastic cells. Likewise, in the case of a pathogen-associated disease, it is

critical to administer a pathogen-specific therapy to avoid any detrimental effect on the non-infected cells.

Chitin, a linear β-1,4 linked polymer of N-acetylglucosamine, is present in the cell walls of all true fungi, but is absent from mammalian cells. Studies in *S. cerevisiae* (reviewed in Bulawa, C., *Mol. Cell. Biol.* 12:1764, 1992; Cabib *et al.*, *Arch. Med. Res.*, 24:301, 1993) have shown that the synthesis of chitin is surprisingly complex, requiring at least three isozymes encoded by the CHS1, CHS2, and CSD2 genes. In cell-free extracts, all of the isozymes catalyze the formation of chitin using UDP-N-acetylglucosamine as the substrate. In cells, each isozyme makes chitin at a unique location in the cell during a specified interval of the cell cycle. Genetic analyses indicate that CHS2 is involved in the synthesis of the chitin-rich primary septum that separates mother and daughter cells, CSD2 is required for synthesis of the chitin rings, and CHS1 plays a role in cell wall repair. Thus, the three isozymes are not functionally redundant and do not substitute for one another.

Chitin synthase genes have been identified from a diverse group of fungi, and analysis of the deduced amino acid sequences of these genes has lead to the identification of two chitin synthase gene families (Bowen, *et al.*, *Proc. Natl. Acad. Sci., USA*, 89:519, 1992). Members of one family are related to the *S. cerevisiae* CHS genes (CHS family). Based on sequence analyses, the CHS family can be subdivided into classes I, II, and III. Members of the second family are related to the *S. cerevisiae* CSD2 gene.

The functions of class II CHS genes have been investigated in a number of fungi by gene disruption. In *S. cerevisiae*, the class II CHS mutant (designated chs2) is defective in cell separation (Bulawa and Osmond, *Proc. Natl. Acad. Sci., USA*, 87:7424, 1990; Shaw *et al.*, *J.*

Cell Biol., 114(1):111, 1990). In *A. nidulans* (Yanai *et al.*, *Biosci.* 58(10):1828, 1994) and *U. maydis* (Gold and Kronstad, *Molecular Microbiology*, 11(5):897, 1994), class II CHS mutants (designated chsA and chs1, respectively) have no obvious phenotype. Thus, all of the class II CHS genes studied to date are nonessential for growth. In addition, Young, *et al.* identified chitin synthase gene which encodes only part of the chitin synthase activity in *C. albicans (Molec. Micro.*, 4(2):197, 1990).

There have been methods designed to identify virulence genes of microorganisms involved in pathogenesis. For example, Osbourn, *et al.* utilized a promoter-probe plasmid for use in identifying promoters that are induced *in vivo* in plants by *Xanthomonas campestris* (*EMBO, J.* 6:23, 1987). Random chromosomal DNA fragments were cloned into a site in front of a promoter-less chloramphenicol acetyltransferase gene contained in the plasmid and the plasmids were transferred into Xanthomonas to form a library. Individual transconjugates were introduced into chloramphenicol-treated seedlings to determine whether the transconjugate displayed resistance to chloramphenicol in the plant.

Knapp, *et al.*, disclosed a method for identifying virulence genes based on their coordinate expression with other known virulence genes under defined laboratory conditions (*J. Bacteriol.*, 170:5059, 1988). Mahan, *et al.*, (U.S. Patent No. 5,434,065) described an *in vivo* genetic system to select for microbial genes that are specifically induced when microbes infect their host. The method depends on complementing the growth of an auxotrophic or antibiotic sensitive microorganism by integrating an expression vector by way of homologous recombination into the auxotrophic or antibiotic sensitive microorganism's chromosome and inducing the expression of a synthetic operon which encodes transcripts, the expression of which

are easily monitored *in vitro* following *in vivo* complementation.

These systems all describe methods of identifying genes involved in pathogenesis in bacterial-host systems. There is a need to identify specific targets of e-ukaryotic pathogens, *e.g.*, fungi, in an infected cell which are associated with the expression of genes whose expression products are implicated in disease, in order to increase efficacy of treatment of infected cells and to increase the efficiency of developing drugs effective against genes essential for survival of these pathogens.

The present invention provides a method for identifying targets essential for growth as well as specific targets identified by the method.

Summary of the Invention

The present invention provides a yeast chitin synthase (CHS1) polypeptide and a polynucleotide encoding the polypeptide. In the present invention,the class II CHS gene of *C. albicans* (encoded by the CHS1 gene) is shown to be essential for growth under laboratory conditions and for colonization of tissues during infection *in vivo*. Thus, CHS1 is a target for the development of antifungal drugs.

CHS1 inhibitors are useful for inhibiting the growth of a yeast. Such CHS1 inhibitory reagents include, e.g., anti-CHS1 antibodies and CHS1 antisense molecules.

CHS1 can be used to determine whether a compound affects (e.g., inhibits) CHS1 activity, by incubating the compound with CHS1 polypeptide, or with a recombinant cell expressing CHS1, under conditions sufficient to allow the components to interact, and then determining the effect of the compound on CHS1 activity or expression.

The invention also provides a vector for identifying a eukaryotic regulatory polynucleotide, including a selectable marker gene; a restriction endonuclease site located at the 5' terminus of the selectable marker gene where a regulatory polynucleotide can be inserted to be operably linked to the selectable marker gene; and a polynucleotide for targeted integration of the vector into the chromosome of a susceptible host. Preferably, the eukaryotic regulatory polynucleotide is a promoter region, and most preferably, a promoter region of pathogenic yeast such as *Candida albicans*. The vector of the invention is preferably transferred to a library of host cells, wherein each host cell contains the vector.

The vector of the invention can be used to identify a eukaryotic regulatory polynucleotide. The method involves inserting genomic DNA of a eukaryotic organism into the vector, wherein the DNA is in operable linkage with the selectable marker gene; transforming a susceptible host with the vector; detecting expression of the selectable marker gene, wherein expression is indicative of operable linkage to a regulatory polynucleotide; and identifying the regulatory polynucleotide.

The vector of the invention also can be used to identify a composition which affects the regulatory DNA (promoter). The method involves incubating the composition to be tested and the promoter, under conditions sufficient to allow the promoter-containing vector of the invention and the composition to interact, and then measuring the effect the composition has on the promoter. The observed effect on the promoter may be either inhibitory or stimulatory.

The method of the invention is useful for identification of promoters from any eukaryote. Particularly preferred eukaryotes are fungal pathogens including, but not limited to, *Candida albicans, Rhodotorula sp., Sac-*

charomyces cerevisiae, Blastoschizomyces capitatus, Histoplasma capsulatum, Aspergillus fumigatus, Coccidioides immitis, Paracoccidioides brasiliensis, Blastomyces dermatitidis, and *Cryptococcus neoformans*.

The invention also features a regulatory polynucleotide (a promoter) isolated using a library of host cells containing the vector of the invention; the promoter is a maltose responsive promoter (MRP), which is induced by maltose and repressed by glucose. MRP is useful for determining whether a polynucleotide encodes a growth-associated polypeptide; the method involves incubating a cell containing the polynucleotide operably linked with the MRP, under conditions which repress the regulatory polynucleotide, and then determining the effect of the expression of the polynucleotide on the growth of the cell.

Brief Description of the Drawings

Figure 1a is a comparison of CHS1 clones.

Figure 1b-g is the nucleotide (SEQ ID NO:1 corresponds to the coding strand and the sequence of SEQ ID NO:3 is complementary to the coding strand) and deduced amino acid sequence (SEQ ID NO.:2) of Chitin Synthase (CHS1) isolated from *Candida albicans*.

Figure 2a is a restriction map of the vector pBluescript® II KS (+/-).

Figure 2b is a restriction map of the vector pVGCA2.

Figure 3a-b is the nucleotide sequence (SEQ ID NO:4) of the maltose responsive promoter (MRP) from *C. albicans* ("X" represents A, G, C, or T/U).

Figure 4 is a schematic illustration showing regulated expression of CHS1 operatively linked to MRP.

Figure 5 is a schematic illustration showing the bidirectional regulation capability of MRP.

Figure 6 is a restriction map of the pKW044 vector including the CHS1 gene.

Figure 7 is a demonstration of gene inactivation during infection by MRP. Panels A and B show neutropenic and Panels C and D show immunocompetent mice infected with the indicated strains of *C. albicans*.

Detailed Description

The invention provides genes essential for growth, such as the chitin synthase gene from *Candida albicans* (CaCHS1), as well as vectors for identification of eukaryotic promoters. Preferably, the vector is used for the identification of promoters of fungal pathogens such as *Candida albicans*. The vectors allow identification of promoters and genes under the control of such promoters, many of which are involved in the infection process. A maltose responsive promoter (MRP) is provided as an example of a promoter isolated using the vector of the invention.

Identification of a yeast gene essential for cell growth

The invention provides a substantially pure chitin synthase (CHS1) polypeptide. The term "substantially pure" as used herein refers to CHS1 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify CHS1 using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the CHS1 polypeptide can also be determined by amino-terminal amino acid sequence analysis. CHS1 polypeptide includes functional fragments of the polypeptide, provided that the activity of CHS1 remains. Smaller peptides containing the biological activity of CHS1 are also included in the invention.

The invention also provides polynucleotides encoding the CHS1 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode CHS1. It is understood that all polynucleotides encoding all or a portion of CHS1 are also included herein, as long as they encode a polypeptide with CHS1 activity. Such polynucleotides include naturally occurring, synthetic, and manipulated polynucleotides. For example, CHS1 polynucleotide may be subjected to site-directed mutagenesis.

The polynucleotide sequence for CHS1 can be used to produce antisense sequences as well as sequences that are degenerate as a result of the degeneracy of the genetic code; there are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention, provided the amino acid sequence of CHS1 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is the yeast CHS1 gene, more specifically, the *Candida albicans* CHS1 gene. The sequence is 3084 base pairs long and contains an open reading frame encoding a polypeptide 1027 amino acids in length and having a molecular weight of about 116kD as determined by reducing SDS-PAGE.

Preferably, the *C. albicans* CHS1 nucleotide sequence is SEQ ID NO:1 and the deduced amino acid sequence is SEQ ID NO:2 (Figure 1b-g).

The polynucleotide encoding CHS1 includes SEQ ID NO:1 as well as nucleic acid sequences capable of hybridizing to SEQ ID NO:1 under stringent conditions. A complementary sequence may include an antisense nucleotide. When the sequence is RNA, the deoxynucleotides A, G, C, and T of SEQ ID NO:1 are replaced by ribonucleotides A, G, C, and U, respectively.

Also included in the invention are fragments of the above-described nucleic acid sequences that are at least 15 bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA that encodes the protein of SEQ ID NO:2 under stringent physiological conditions.

The CHS1 polypeptide of the invention can be used to produce antibodies which are immunoreactive with or which specifically bind to epitopes of the CHS1 polypeptide. As used herein, the term "epitope" means any antigenic determinant of an antigen to which an antibody to the antigen binds.

Antibodies can be made to the protein of the invention, including monoclonal antibodies, which are made by methods well known in the art (Kohler, *et al.*, *Nature*, 256:495, 1975; *Current Protocols in Molecular Biology, Ausubel, et al.*, ed., 1989).

The term "antibody" as used in this invention includes intact molecules as well as fragments thereof, such as Fab, F(ab')2, and Fv which are capable of binding the epitopic determinant. These antibody fragments retain the ability to selectively bind with its antigen or receptor and are defined as follows: (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule; (3) (Fab')2, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent

reduction; F(ab')2 is a dimer of two Fab' fragments held together by two disulfide bonds; (4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. Methods of making these fragments are known in the art. (See for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1988), incorporated herein by reference).

Antibodies which bind to the CHS1 polypeptide of the invention can be prepared using an intact polypeptide or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or a peptide used to immunize an animal can be derived from transcribed/translated cDNA or chemical synthesis, and can be conjugated to a carrier protein, if desired. Such commonly used carriers which can be chemically coupled to the peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is used to immunize the animal (*e.g.*, a mouse, a rat, or a rabbit).

It is also possible to use the anti-idiotype technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the "image" of the epitope bound by the first monoclonal antibody.

The invention also provides a method for inhibiting the growth of yeast, by contacting the yeast

with a reagent which suppresses CHS1 activity. Preferably the yeast is *C. albicans*.

Where a disease or disorder is associated with the production of CHS1 (*e.g.*, a yeast infection), nucleic acid sequences that interfere with CHS1 expression at the translational level can be used to treat the infection. This approach utilizes, for example, antisense nucleic acids, ribozymes, or triplex agents to block transcription or translation of CHS1 mRNA, either by masking that mRNA with an antisense nucleic acid or triplex agent or by cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA, as the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the CHS1-producing cell (*e.g.*, a *Candida albicans*). The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal.Biochem.*, 172:289, 1988).

Use of an oligonucleotide to block transcription is known as the triplex strategy; the oligomer winds around double-helical DNA, forming a three-strand helix. These triplex compounds can be designed to recognize a unique site on a chosen gene (Maher, *et al.*, *Antisense Res. and Dev.*, 1(3):227, 1991; Helene, C., *Anticancer Drug Design*, 6(6):569, 1991).

The reagent used for inhibition of the growth of yeast by suppression of CHS1 activity can be an anti-CHS1 antibody. Addition of such an antibody to a cell or tissue suspected of containing a yeast, such as *C. albicans*, can prevent cell growth by inhibiting cell wall formation.

The invention also provides a method for detecting a yeast cell in a host tissue, for example, which comprises contacting an anti-CHS1 antibody or CHS1 polynucleotide with a cell having a yeast-associated infection and detecting binding to the antibody or hybridizing with the polynucleotide, respectively. The antibody or polynucleotide reactive with CHS1 or DNA encoding CHS1 is labeled with a label which allows detection of binding or hybridization to CHS1 or the DNA. An antibody specific for CHS1 polypeptide or polynucleotide specific for CHS1 polynucleotide may be used to detect the level of CHS1 in biological fluids and tissues of a patient.

The antibodies of the invention can be used, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier.

The anti-CHS1 antibodies of the invention can be bound to a solid support and used to detect the presence of an antigen of the invention. Examples of well-known supports include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

The CHS1 antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of

amelioration of a yeast-associated disease in a subject. Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising CHS1 polypeptide of the invention or changes in the concentration of such antigen present in various body fluids, it is possible to determine whether a particular therapeutic regimen aimed at ameliorating the yeast-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the yeast-associated disease in the subject receiving therapy.

The CHS1 of the invention is also useful in a screening method to identify compounds or compositions which affect the activity of the protein. To determine whether a compound affects CHS1 activity, the compound is incubated with CHS1 polypeptide, or with a recombinant cell expressing CHS1, under conditions sufficient to allow the components to interact; the effect of the compound on CHS1 activity or expression is then determined.

The increase or decrease of chitin synthase transcription/translation can be measured by adding a radioactive compound to the mixture of components, such as ^{32}P-ATP or ^{35}S-Met, and observing radioactive incorporation into CHS1 transcripts or protein, respectively. Alternatively, other labels may be used to determine the effect of a composition on CHS1 transcription/translation. For example, a radioisotope, a fluorescent compound, a bioluminescent compound, a c-hemiluminescent compound, a metal chelator or an enzyme could be used. Those of ordinary skill in the art will know of other suitable labels or will be able to ascertain such, using routine experimentation. Analysis of the effect of a compound on CHS1 is performed by standard methods in the art, such as Northern blot

analysis (to measure gene expression)or SDS-PAGE (to measure protein product), for example. Further, CHS1 enzymatic activity can also be determined, for example, by incorporation of labeled precursor of chitin. Preferably, such precursor is UDP-N-acetylglucoseamine.

Vector for identification of a eukaryotic regulatory polynucleotide

The vector contains at least one promoterless selectable marker gene and a restriction endonuclease cloning site located at the 5′ terminus of the selectable marker. A pool of chromosomal DNA fragments from a eukaryotic organism is inserted at the restriction endonuclease cloning site in operable linkage with the selectable marker polynucleotide. In addition, the vector contains a polynucleotide sequence for targeted integration of the vector into the chromosome of a susceptible host.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid, to which it has been operatively linked, from one genetic environment to another.

The term "regulatory polynucleotide" as used herein preferably refers to a promoter, but can also include enhancer elements. The vectors of the invention contain a promoterless selectable marker gene having a cloning site at the 5′ terminus of the gene. The vectors also include a cloning site 5′ of the selectable marker gene, which is operably associated with a promoter. The term "operably associated" or "operably linked" refers to functional linkage between the promoter sequence and the controlled nucleic acid sequence; the sequence and promoter are typically covalently joined, preferably by conventional phosphodiester bonds.

The expression vectors of the invention employ a promoterless gene for selection of a promoter sequence. The vectors contain other elements typical of vectors, i-ncluding an origin of replication, as well as genes which are capable of providing phenotypic selection of transformed cells. The transformed host cells can be grown in the appropriate media and environment, *e.g.*, in fermentors, and cultured according to techniques known in the art to achieve optimal cell growth. The vectors of the present invention can be expressed *in vivo* in either prokaryotes or eukaryotes. Methods of expressing DNA sequences containing eukaryotic coding sequences in prokaryotes are well known in the art. Biologically functional plasmid DNA vectors used to incorporate DNA sequences of the invention for expression and replication in the host cell are described herein. For example, DNA can be inserted into yeast cells using the vectors of the invention. Various shuttle vectors for the expression of foreign genes in yeast have been reported (Heinemann, *et al.*, *Nature*, 340:205, 1989; Rose, *et al.*, *Gene*, 60:237, 1987).

Host cells include microbial, yeast, and mammalian cells, e.g., prokaryotes and eukaryotes such as yeast, filamentous fungi, and plant and animal cells.

Transformation or transfection with recombinant DNA may be carried out by conventional techniques well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after the exponential growth phase and subsequently treated, i.e., by the $CaCl_2$ method using procedures well known in the art.

Where the host cell is eukaryotic, various methods of DNA transfer can be used. These include transfection of DNA by calcium phosphate-precipitates, conventional

mechanical procedures such as microinjection, insertion of a plasmid encased in liposomes, spheroplast, electroporation, salt mediated transformation of unicellular organisms, or the use of viral vectors. A library of host cells, wherein each host cell contains a vector according to the description above, is also included in the invention.

Eukaryotic DNA can be cloned into prokaryotes using vectors well known in the art. Because there are many functions in eukaryotic cells which are absent in prokaryotes, (e.g., localization of ATP-generating systems to mitochondria, association of DNA with histone, mitosis and meiosis, and differentiation of cells), the genetic control of such functions must be assessed in a eukaryotic environment. Many eukaryotic vectors, though, are capable of replication in *E. coli*, which is important for amplification of the vector DNA. Thus, vectors preferably contain markers, *e.g.*, LEU 2, HIS 3, URA 3, that can be selected easily in yeast, and in addition, also carry antibiotic resistance markers for use in *E. coli*. The selectable marker gene, which lies immediately downstream from the cloning site, preferably encodes a biosynthetic pathway enzyme of a eukaryote which relies on the enzyme for growth or survival. This biosynthetic pathway gene, once activated, will complement the growth of an auxotrophic host, deficient for the same biosynthetic pathway gene in which it is integrated. Typically, genes encoding amino acid biosynthetic enzymes are utilized, since many strains are available having at least one of these mutations, and transformation events are easily selected by omitting the amino acid from the medium. Examples of markers include but are not limited to URA3, URA3-hisG, LEU2, LYS2, HIS3, HIS4, TRP1, ARG4, Hgm^R, and TUN^R. Preferably, the vector includes a promoterless URA3 gene. Expression of the *C. albicans*

URA 3 gene is required for the infection process, thus creating a strong selection pressure for those sequences cloned upstream of the promoterless URA3 gene that will be induced during the infection process.

The vector of the invention preferably includes a prokaryotic origin of replication or replicon, *i.e.*, a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally in a transformed prokaryotic host cell. Such origins of replication are well known in the art; preferred origins of replication are those that are efficient in the host organism, e.g., the preferred host cell, *E. coli*. For vectors used in *E. coli*, a preferred origin of replication is ColE1, which is found in pBR322 and a variety of other common plasmids. Also preferred is the p15A origin of replication found on pACYC and its derivatives. The ColE1 and p15A replicon have been extensively utilized in molecular biology, are available on a variety of plasmids, and are described, e.g., in Sambrook, *et al.*, *Molecular Cloning: a Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, 1989).

The ColE1 and p15A replicons are particularly preferred for use in the invention because they each have the ability to direct the replication of a plasmid in *E. coli* while the other replicon is present in a second plasmid in the same *E. coli* cell. In other words, ColE1 and p15A are non-interfering replicons that allow the maintenance of two plasmids in the same host (see, for example, Sambrook, *et al.*, *supra*, at pages 1.3-1.4).

The vector of the invention includes a polylinker multiple cloning site for insertion of selectable marker genes. A sequence of nucleotides adapted for directional ligation, *i.e.*, a polylinker, is a region of the DNA expression vector that (1) operatively links for

replication and transport the upstream and downstream translatable DNA sequences, and (2) provides a site for directional ligation of a DNA sequence into the vector. Typically, a directional polylinker is a sequence of nucleotides that defines two or more restriction endonuclease recognition sequences. Upon restriction cleavage, the two sites yield cohesive termini to which a translatable DNA sequence can be ligated to the DNA expression vector. Preferably, the two restriction sites provide, upon restriction cleavage, cohesive termini that are non-complementary and thereby permit directional insertion of a translatable DNA sequence into the cassette. Where the sequence of nucleotides adapted for directional ligation defines numerous restriction sites, it is referred to as a multiple cloning site.

Additionally, the vector may contain a phenotypically selectable marker gene to identify host cells which contain the expression vector. Examples of markers typically used in prokaryotic expression vectors include antibiotic resistance genes for ampicillin (β-lactamases), tetracycline and chloramphenicol (chloramphenicol acetyltransferase).

The vector contains a polynucleotide sequence for targeted integration of the vector into the chromosome of a susceptible host. Targeted integration, as opposed to random integration, results in more stable transformants and avoids position effects or integration into genes required for growth and infection. Preferably, the gene for targeted integration is also a selectable marker, thereby allowing the identification of transformants that contain the vector. Such genes include the adenine biosynthesis(ADE2) gene of *Candida albicans*. A susceptible host is a host having a site recognized by the polynucleotide of the vector for targeted integration.

Promoters identified by the method of the invention can be inducible or constitutive promoters. Inducible promoters can be regulated, for example, by nutrients (*e.g.*, carbon sources, nitrogen sources, and others), drugs (*e.g.*, drug resistance), environmental agents that are specific for the infection process (*e.g.*, serum response), and temperature (*e.g.*, heat shock, cold shock).

<u>Identification of a eukaryotic regulatory polynucleotide</u>

The selection method of the invention utilizes an auxotrophic organism, or an organism that has a mutation in a biosynthetic pathway gene encoding a functional biosynthetic enzyme necessary for the growth of the organism. When a functional or wild-type copy of a biosynthetic pathway gene is inserted into an auxotroph, the expression of the wild-type biosynthetic pathway gene provides the auxotroph with the biosynthetic enzyme required for growth or survival. The process of replacing a missing or non-functional gene of an auxotroph with a functional homologous gene in order to restore the auxotroph's ability to survive within a host cell is called "complementation".

Complementation of the auxotroph, according to the present invention, is accomplished by construction of a vector having a promoterless structural gene encoding a biosynthetic enzyme, *i.e.*, a selectable marker polynucleotide, as described above. The cloning site for the promoter of interest is at the 5' terminus of the structural gene encoding the biosynthetic enzyme. Consequently, a promoter region operatively linked to any gene or set of genes will control the expression of that gene or genes. In order to be controlled by the promoter, the gene must be positioned downstream from the promoter.

The structural gene encoding a biosynthetic enzyme in the vector of the invention does not contain recognition sequences for regulatory factors to allow transcription of the structural gene. Consequently, the product(s) encoded by the structural gene is not capable of being expressed unless a promoter sequence is inserted into the cloning site 5′ to the structural gene.

A second structural gene in the vector allows for targeted insertion and integration into the host cell's chromosomal DNA. Optionally, the vector may contain additional genes, such as those encoding selective markers for selection in bacteria. Typically drug resistance genes such as those described above are used for such selection.

In the method of the invention, total genomic DNA is isolated from the organism, *e.g.*, *Candida albicans*, and then partially enzymatically digested, resulting in a pool of random chromosomal fragments. The vector of the invention is cleaved at the restriction/cloning site, and mixed with the cleaved chromosomal DNA. The chromosomal fragments are ligated into the vector to produce a library, *i.e.*, each vector contains a random chromosomal fragment so that the pool of vectors is representative of the entire organism's genome. The vectors containing the chromosomal fragments are then introduced into the host organism (*e.g.*, an auxotrophic strain or drug resistant strain of *Candida albicans*) by methods well know in the art. For example, the vectors may be introduced by t-ransformation.

After the vector is introduced into the host (*e.g.*, auxotrophic), the vector may integrate into the auxotroph's chromosome by targeted integration. This step can be detected by selection, as described above. For example, the preferred polynucleotide for targeted insertion and integration in *Candida albicans* is the ADE2

gene. The presence of this gene is detectable by growth of the organism on adenine deficient media.

The expression of the biosynthetic enzyme gene, *e.g.*, URA3, whether under constitutive or inducible conditions, is identified by complementation of a host cell strain in which the gene is defective or missing, *e.g.*, URA3-. Only those host cells which can grow in medium lacking the nutritional supplement, *e.g.*, uracil, will be expected to contain a cloned functional promoter sequence.

Identification of a yeast regulatory polynucleotide capable of induction and repression

In another aspect, the invention provides an isolated regulatory polynucleotide, the MRP promoter, characterized in that it is induced by maltose and repressed by glucose. MRP of the invention is exemplified by the nucleotide sequence of SEQ ID NO:4 (Figure 3a-b), wherein the sequence is 1734 base pairs in length. MRP was isolated from a promoter library based on expression of the Ura3 gene of *C. albicans* as described above. MRP functions bidirectionally, that is, genes flanking MRP both 5′ and 3′ are controlled by this regulatory polynucleotide.

The MRP of the invention is useful for identifying genes which are essential for cell growth. Thus, the invention provides a method for determining whether a polynucleotide encodes a growth-associated polypeptide, by incubating a cell containing the polynucleotide operably linked with the MRP regulatory polynucleotide, under conditions which repress the regulatory polynucleotide, and determining the effect of the tested polynucleotide on the growth of the cell.

MRP of the invention promotes transcription in the presence of maltose, while the ability of MRP to promote

transcription is repressed by glucose. A cell having a polynucleotide of interest operably linked to MRP can be grown on a glucose containing medium to determine whether the polynucleotide of interest is essential for cell growth. MRP is repressed on glucose, thus repressing transcription of the operably linked polynucleotide, therefore, if a cell grown on a glucose containing-medium dies, the polynucleotide is determined to be essential for cell growth.

MRP can be used to induce (maltose) or repress (glucose) expression of a gene operably linked to MRP. It is also envisioned that MRP may be useful for decreasing the expression of a target gene operably linked to MRP, such that the cell containing the MRP-gene of interest is now extremely sensitive to a compound of interest. For example, it may be desirable to increase susceptibility or resistance to a particular therapeutic compound. - Similarly, MRP is useful for inducing expression of a gene operatively linked to MRP, by growing a host cell containing a MRP-gene construct on a maltose-containing medium. It may be desirable to elevate gene expression for screening various therapeutic compounds for their effect on the gene product.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

EXAMPLES

EXAMPLE 1

ISOLATION OF CHITIN SYNTHASE FROM *Candida albicans*

Using Southern blotting, the restriction maps for the cloned CHS1 gene contained in pJAIV and the genomic CHS1 locus were produced, however, the maps were found not to match. Additional studies indicated that pJAIV

contained two nonadjacent genomic DNA fragments as diagrammed in FIGURE 1a. As a consequence, pJAIV lacked the 5′ end of CHS1. To clone this region, a plasmid rescue strategy was employed. Plasmid pKW025, which contains a 600 bp *Kpn*I/*Eco*RI fragment of CHS1, and a 1.4 kb Candida URA3 gene cloned into pSK(-), was cut with *Cla*I and transformed into *Candida albicans* strain CAI-4. Transformants were examined by Southern blot and strain CAI-4A was identified, containing pKW025 integrated at the CHS1 locus. Genomic DNA was extracted from CA1-4A and cut with *Hind* III. Because pKW025 and the sequenced portion of CHS1 contain no *Hind* III sites, this digestion yields on a single DNA fragment pKW025 plus the genomic CHS1 locus with flanking regions extending to the 5′ and 3′ *Hind* III sites. Ligation was carried out with a low DNA concentration to promote intramolecular ligation events, and the DNA transformed into *E. coli*. Recovered plasmids were screened by PCR to verify that they contained contiguous CHS1 sequence.

Plasmid pKW030 (12 kb total) was identified and contained approximately 2 kb of CHS1 sequence upstream of the *Xho*I site. A 3.6 kb *Hind*III/*Pst*I fragment was cloned into the *Hind*III/*Pst*I sites of pSK(-), forming plasmid pKW032. The 3′ region of the gene was derived from plasmid pKW013 (originally derived from pJA-IV). A 3.5 kb *Bst*EII/*Not*I fragment was cloned into the *Bst*EII/*Not*I sites of pKW032, forming plasmid pKW035. pKW035 was cut with various restriction enzymes, and Southern blot analysis also carried out to confirm that the insert was indeed an uninterrupted CHS1 gene whose restriction pattern matched that of the chromosomal CHS1.

The insert was sequenced by standard methods and the nucleotide and deduced amino acid sequence are shown in Figure 1b-g (SEQ ID NO:1 and 2).

EXAMPLE 2

CONSTRUCTION OF PROMOTER ISOLATION VECTOR

The *Candida albicans* URA3 gene was amplified by PCR and a *Sal*I site was inserted next to the ATG. The 3′ primer used contained a genomic *Xba*I site. The *Sal*I/*Xba*I fragment was cloned in Bluescript KS+ at *Sal*I/*Xba*I. The *C. albicans Eco*RV genomic fragment containing the ADE2 gene was cloned in the above plasmid at the *Xho*I site of the Bluescript polylinker.

The Ca URA3 gene was amplified by PCR using the following primers:

5′ Primer URA3-ATG: 5′-GGAGGA[GTCGAC]ATGACAGTCAACAC-3′
*Sal*I
(SEQ ID NO:5)

3′ Primer URA3-*Xba*I: 5′-CGCATTAAAGC[TCTAGA]AGAACCACC-3′
*Xba*I
(SEQ ID NO:6)

(Underlined regions: genomic)

The PCR reaction was as follows:
100 ng DNA, 50pmoles each primer, 2.5mM dNTP, 2.5mM Mg Cl_2, 0.5U Taq Polymerase/100 µl.

Reaction:

step 1: 2 min 94°C
step 2: 1 min 94°C
step 3: 1 min 57°C
step 4: 11/2 min 72°C
step 5: steps 2-4 x 30 times
step 6: 10 min 72°C
step 7: Hold 4°C

For the cloning, 20 µl of the PCR reaction was run on 0.7% low melting agarose gel and the band was purified using the Promega (Madison, WI) PCR purification resin. The purified band and 1 µg of Strategene KS+ bluescript (Figure 2a; Stratagene, La Jolla, CA) were digested with

*Sal*I and *Xba*I, gel isolated (as above) and eluted in 50 μl water.

The ligation reaction was performed as follows: Ligation (20 μl): 1 μl vector, 10 μl digested PCR band, 2 μl T4 ligase buffer, 1 μl (2 units) T4 ligase (Boehrringer), 6 μl H_2O, over night at room temperature. 10 μl of the ligation was used to transform Strategene XL1 Blue ultracompetent cells selecting for ampicillin resistance. Individual colonies were grown in LB+ ampicillin and plasmid DNA was isolated using the Quiagen (Chatsworth, CA) spin columns.

The above plasmid was digested with *Xho*I, filled in with Klenow for 30 min and dephosporylated with acid phosphatase for 5 min. The band was gel purified as above. The *Eco*RV fragment containing the Ca ADE2 gene was cloned into the plasmid using the conditions described above(Figure 2b).

EXAMPLE 3

Isolation and Characterization of a maltose induced/glucose repressed promoter of *C. albicans*

Using the promoter probe vector pVGCAV2 (based on *URA*3 expression), a library was constructed which inserted 1-2 kb *Sau*3A fragments (isolated by sucrose gradient centrifugation) upstream (5′) of the promoterless *URA*3 reporter gene into the vector. The vector plasmid was cut with *Sal*I and partially end filled with dT and dC while the insert fragments (*Sau*3A cut) were partially filled in with dG and dA. These partial fill in reactions left 2 bp overhangs that are compatible for a ligation reaction. The results of the ligation of the library were introduced into *E. coli* strain DH5α by electroporation, and gave rise to 76,500 independent transformants. Sixteen randomly picked colonies all proved to have inserts indicating the library was sound.

The plasmid library was extracted from *E. coli* by standard plasmid isolation procedures and cut at the unique *Bam*HI site within the *ADE*2 gene for targeted integration of the *ADE* locus of *C. albicans* strain CaI8 (*ade2ura3*). The *ade*2 mutation of CaI8 allows for selection of transformants and the *ura*3 mutation of CaI8 permits monitoring of expression of the reporter gene *URA*3. A first pool of 10,000 independent CaI8 transformants was tested for regulated *URA*3 expression. The CaI8 transformants were plated on Synthetic Dextrose [glucose medium (2% glucose (w/v) and yeast nitrogen base without amino acids at 6.7 g/L (Difco)) without uridine] to determine the frequency of transformants expressing the *URA*3 gene constitutively. Fourteen per cent of the Candida CaI8 transformants expressed varying levels of the *URA*3 gene as determined by the ability to form colonies on a medium lacking uridine supplementation. The pool was then treated with the compound 5-FOA to remove these transformants expressing the *URA*3 gene constitutively (transformants expressing *URA*3 convert 5-Fluoro-orotic acid to a toxic compound and thus can be eliminated from the pool). To isolate promoters responding to specific carbon sources, aliquots of the pool were grown on synthetic glucose medium supplemented with uridine and replicated to synthetic maltose medium without uridine. *Candida* transformants able to produce colonies on the unsupplemented maltose medium putatively contained a maltose inducible promoter. Four strains (MRP-2, MRP-5, MRP-6, MRP-7) were shown to show maltose dependent growth that was repressed upon the addition of glucose.

Chromosomal DNA was extracted from the *Candida* CaI8 transformants exhibiting maltose dependent growth (MRP strains) and digested with the restriction enzyme *Bam*HI to "release the MRP clones." The "released"

plasmids were ligated and introduced into *E. coli* by transformation. These *E. coli* transformants were used as a source of plasmid DNA for dideoxy/chain termination sequencing. Initial sequencing data using a primer to *URA*3 sequences just downstream of the insert (3′) indicated all the MRP strains contained the same insert. Sequencing data obtained using a primer to *ADE*2 sequences (5′ to the insert DNA with respect to *URA*3 transcription indicated the clone contained part of a maltase gene and regulatory sequences (Figure 3a-b, SEQ ID NO:4). The entire sequence of the clone was assembled and the portion of the maltase ORF contained on the insert was shown to be approximately 70% sequence identical to a previously cloned promoter of *C. albicans* maltase (CAMAL2) (Geber, *et al., J. Bacteriology, 174*:6992, 1992).

EXAMPLE 4

IDENTIFICATION OF GENES ESSENTIAL FOR YEAST CELL GROWTH

This experiment used the MRP promoter as a gene disruption tool, and the *C. albicans CHS*1 gene. A strain was constructed and designated KWC340, in which *CHS*1 expression is regulated by the carbon source present in the growth medium. Transcription of *CHS*1 was induced by maltose and repressed by glucose. In maltose containing medium, KWC340 grows at the same rate as a wild-type strain. When KWC340 is transferred to glucose-containing medium, cells stop growing and eventually die. Three generations after transfer to glucose, short chains of cells grow but fail to separate. Ten generations after transfer, growth has stopped. Long chains and clumps of cells are seen; a large percentage of the cells are anucleate or multinucleate, indicating a defect in nuclear segregation. Viability is reduced approximately

500-fold relative to a control culture, as judged by plating efficiency.

As a first step in constructing a strain in which the sole functional *CHS*1 gene was under the control of the MRP fragment, a vector was constructed in pKS termed KWO44 with the following features (see Figure):

(a) the plasmid contained *URA*3 for selection of transformants in the Ura-strains CaI4 (*CHS*1/*CHS*1) and 167b (*CHS*1/*chs*1::hisG)

(b) a 1088 bp PCR fragment of the MRP sequence (see attached figure showing sites of PCR primers)

(c) 1479 bp of the *C. albicans CHS*1 N-terminus that contains a unique XhoI site to target the transformation/integration event.

This construct fuses the ATG initiation codon of the *CHS*1 gene at the same position as the *URA*3 gene (original reporter gene used to isolate the MRP clone) with respect to the MRP fragment. Integration of this construct at the remaining wild-type *CHS*1 allele in strain 167b places the sole functional *CHS*1 gene under the control of the transcriptional control of the MRP fragment. After transformation this type of integrants were recovered as confirmed by Southern analysis. These integrants grew well on maltose containing medium (inducing conditions) but died when replicated to glucose containing medium.

When injected into mice, the MRP-CHS1 integrants were avirulent;the symptoms diagnostic of candidiasis were not observed, and the kidneys from the mice were sterile. Thus CHS1 is essential for growth *in vitro* and *in vivo*. Briefly, ICR 4-week-old male mice (Harlan Sprague Dawley) were housed five per cage; food and water were given ad libitum according to the National Institutes of Health guidelines for the ethical treatment of animals. Strains of *C. albicans* were grown in SM

medium [2% maltose, 0.7% yeast nitrogen base without amino acids (Difco Laboratories, Detroit, MI)] to a density of 10^7 cells/ml. Cells were harvested, washed, resuspended in sterile water, and injected into mice (10^6 cells/immunocompetent mouse, 10^4 cells/neutropenic mouse) via the lateral tail veins. For each strain of *C. albicans*, five mice were infected. Cages were checked three times daily for mice dead or moribund (exhibiting severe lethargy, vertigo, and ruffled fur) mice. Moribund mice were euthenized by cervical dislocation and necropsied. The left and right kidneys were removed and examined for colonization by *C. albicans*. In experiments using neutropenic mice, cyclophosphamide was administered (150 mg/kg) by intraperitoneal injection 96 and 24 hours prior to infection. Injections were repeated every three days for the duration of the experiment. Neutropenia was verified by comparing the percentage of neutrophils to total number of leukocytes before and after injection with cyclophosphamide.

Figure 7, panels A-D, shows the results of the *in vivo* experiment. Neutropenic (panels A & B) and immunocompetent (panels C & D) mice were infected with the indicated strains of *C. albicans*: clinical isolate (strain SC5314, , panels A & C); MRP::URA3 (strain MRP2, a derivative of SC5314 containing one copy of URA3 which is regulated by MRP, □, panels A & C); MRP::CHS1 (strain KWC340, a derivative of SC5314 containing one copy of CHS1 which is regulated by MRP, △, panels B & D); and CHS1/MRP::CHS1 (strain KWC352, a derivative of SC5314 containing two copies of CHS1; one regulated by MRP, the other by the CHS1 promoter, O, panels B & D).

In conclusion, these results show the MRP clone controls the expression of two non cognate genes (*CHS*1 and *URA*3) in a regulated manner and demonstrate the utility of the MRP sequence as a genetic tool in *C.*

albicans for target validation (determination of gene essentiallity).

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: CHEMGENICS PHARMACEUTICALS, INC.

(ii) TITLE OF THE INVENTION: IDENTIFICATION OF EUKARYOTIC GROWTH-RELATED GENES AND PROMOTER ISOLATION VECTOR AND METHOD OF USE

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Boston
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 01-NOV-1996
(C) CLASSIFICATION:

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(B) FILING DATE: 01-NOV-1995

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3084 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 1...3081
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATG AAG AAT CCA TTT GAC AGT GGC AGT GAC GAT GAA GAT CCA TTT CTT       48
Met Lys Asn Pro Phe Asp Ser Gly Ser Asp Asp Glu Asp Pro Phe Leu
 1               5                  10                  15

AGT AAT CCA CAA TCT GCA CCA TCA ATG CCC TAC GCA GCA TAT TTC CCA       96
Ser Asn Pro Gln Ser Ala Pro Ser Met Pro Tyr Ala Ala Tyr Phe Pro
            20                  25                  30

CTG TCG ACT AGT GGA TCT CCA TTT CAC CAA CAG CAA TCC CCA AGA CAA      144
Leu Ser Thr Ser Gly Ser Pro Phe His Gln Gln Gln Ser Pro Arg Gln
        35                  40                  45

TCA CCT AAT ATT TTT TCC AGA AGT ACT GCA AGA GCA ACT AGT GAC AGA      192
Ser Pro Asn Ile Phe Ser Arg Ser Thr Ala Arg Ala Thr Ser Asp Arg
    50                  55                  60

ACA TCG CCC CGC AAG ACA TAC CAA CCA TTG AAT TTT GAC AGT GAG GAC      240
Thr Ser Pro Arg Lys Thr Tyr Gln Pro Leu Asn Phe Asp Ser Glu Asp
65                  70                  75                  80

GAA GAT GCT AAA GAA AGC GAA TTT ATG GCT GCA ACC TCA AAG CTG AAT      288
Glu Asp Ala Lys Glu Ser Glu Phe Met Ala Ala Thr Ser Lys Leu Asn
                85                  90                  95

ATG AGC ATA TAT GAT AAT ACC CCG AAC TTA CAA TTC AAC AAA AGC GGC      336
Met Ser Ile Tyr Asp Asn Thr Pro Asn Leu Gln Phe Asn Lys Ser Gly
            100                 105                 110

GCA GCC ACA CCA AGA GCA CAA TTC ACA TCG AAA GAA TCT CCG AAA AGA      384
Ala Ala Thr Pro Arg Ala Gln Phe Thr Ser Lys Glu Ser Pro Lys Arg
        115                 120                 125

CAA AAA ACT ACT GAA GTG ACC ATT GAC TTT GAC AAT GAT GAT GAT AAC      432
Gln Lys Thr Thr Glu Val Thr Ile Asp Phe Asp Asn Asp Asp Asp Asn
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AAT CAC ACC TTA GAA TTT GAA AAT GGG TCA CCT CGT CGT TCA TTT CGT      480
Asn His Thr Leu Glu Phe Glu Asn Gly Ser Pro Arg Arg Ser Phe Arg
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Ser Ser Ala Ile Ser Ser Glu Arg Phe Leu Pro Pro Pro Gln Pro Ile
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        195                 200                 205

CAG AAG GGT TAT GAG GAA GTA GAA ACA TTG CAT TCG GAA GGT ACA GCT      672
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260 265 270

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Arg Leu Val Gly Gly Lys Ala Gly Asn Leu Val Leu Glu Asn Pro Val
275 280 285

CCA ACA GAG TTG AGA AAA GTG TTG ACC AGA ACC GAG TCT CCA TTT GGT 912
Pro Thr Glu Leu Arg Lys Val Leu Thr Arg Thr Glu Ser Pro Phe Gly
290 295 300

GAG TTT ACC AAC ATG ACA TAC ACA GCG TGC ACT TCG CAG CCA GAT ACT 960
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325 330 335

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340 345 350

TTT GCC AGA ACT ATG CAT GGT GTG ATG AAA AAT ATC GCT CAT TTG TGC 1104
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355 360 365

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CCC TAT GTC AAC AAT AGC AAA GTA AAT GCC CAT TTG TTT GAA TAT ACC 1296
Pro Tyr Val Asn Asn Ser Lys Val Asn Ala His Leu Phe Glu Tyr Thr
420 425 430

ACT CAA ATA TCT ATC GAT GAG AAC TTG AAA TTC AAA GGA GAT GAA AAA 1344
Thr Gln Ile Ser Ile Asp Glu Asn Leu Lys Phe Lys Gly Asp Glu Lys
435 440 445

AAC CTT GCA CCA GTT CAA GTC TTG TTC TGT TTG AAA GAA CTG AAC CAA 1392
Asn Leu Ala Pro Val Gln Val Leu Phe Cys Leu Lys Glu Leu Asn Gln
450 455 460

AAG AAA ATC AAT TCC CAT AGA TGG CTT TTT AAT GCC TTT TGT CCT GTC 1440
Lys Lys Ile Asn Ser His Arg Trp Leu Phe Asn Ala Phe Cys Pro Val
465 470 475 480

TTG GAC CCC AAT GTT ATT GTT CTT TTA GAT GTG GGT ACC AAA CCC GAT 1488
Leu Asp Pro Asn Val Ile Val Leu Leu Asp Val Gly Thr Lys Pro Asp
485 490 495

AAC CAT GCC ATT TAT AAT CTA TGG AAA GCA TTC GAT AGA GAT TCC AAT 1536
Asn His Ala Ile Tyr Asn Leu Trp Lys Ala Phe Asp Arg Asp Ser Asn
500 505 510

GTA GCA GGG GCT GCT GGT GAA ATT AAA GCG ATG AAA GGT AAA GGT TGG 1584
Val Ala Gly Ala Ala Gly Glu Ile Lys Ala Met Lys Gly Lys Gly Trp
515 520 525

ATT AAT CTT ACA AAT CCA TTA GTT GCG TCA CAG AAT TTT GAG TAT AAA 1632
Ile Asn Leu Thr Asn Pro Leu Val Ala Ser Gln Asn Phe Glu Tyr Lys
530 535 540

TTG TCC AAT ATT CTT GAT AAA CCG TTG GAA TCA CTT TTT GGA TAC ATT 1680
Leu Ser Asn Ile Leu Asp Lys Pro Leu Glu Ser Leu Phe Gly Tyr Ile
545 550 555 560

TCT GTG TTA CCA GGT GCA TTG TCT GCA TAT CGA TAC ATT GCC TTG AAA 1728
Ser Val Leu Pro Gly Ala Leu Ser Ala Tyr Arg Tyr Ile Ala Leu Lys
565 570 575

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580 585 590

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Asp Leu Leu Cys Ser His Asp Lys Asp Lys Glu Asn Thr Lys Ala Asn
595 600 605

TTT TTC GAA GCA AAT ATG TAC TTG GCT GAA GAC AGA ATC CTT TGT TGG 1872
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610 615 620

GAA TTG GTA TCA AAA AGA AAT GAC AAT TGG GTT CTT AAA TTT GTT AAA 1920
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625 630 635 640

CTG GCA ACC GGT GAA ACT GAT GTT CCT GAA ACA ATT GCA GAA TTT CTT 1968
Leu Ala Thr Gly Glu Thr Asp Val Pro Glu Thr Ile Ala Glu Phe Leu
645 650 655

TCG CAA AGA CGA AGA TGG ATT AAT GGT GCC TTT TTT GCT GCT TTG TAC 2016
Ser Gln Arg Arg Arg Trp Ile Asn Gly Ala Phe Phe Ala Ala Leu Tyr
660 665 670

TCC TTG TAT CAC TTT AGA AAA ATA TGG ACG ACT GAC CAT TCG TAT GCT 2064
Ser Leu Tyr His Phe Arg Lys Ile Trp Thr Thr Asp His Ser Tyr Ala
675 680 685

AGA AAA TTT TGG CTA CAT GTC GAA GAA TTC ATT TAT CAA TTG GTA TCA 2112
Arg Lys Phe Trp Leu His Val Glu Glu Phe Ile Tyr Gln Leu Val Ser
690 695 700

TTA TTG TTT TCA TTT TTT TCT TTG AGT AAT TTC TAT TTA ACA TTT TAT 2160
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705 710 715 720

TTT TTG ACA GGT TCA TTG GTG TCT TAC AAA AGT CTT GGT AAA AAA GGT 2208
Phe Leu Thr Gly Ser Leu Val Ser Tyr Lys Ser Leu Gly Lys Lys Gly
725 730 735

GGA TTT TGG ATT TTC ACA TTA TTC AAT TAT CTC TGT ATC GGT GTT TTG 2256
Gly Phe Trp Ile Phe Thr Leu Phe Asn Tyr Leu Cys Ile Gly Val Leu
740 745 750

ACA TCT TTG TTC ATT GTC TCC ATT GGT AAT AGA CCA CAT GCA TCA AAG 2304
Thr Ser Leu Phe Ile Val Ser Ile Gly Asn Arg Pro His Ala Ser Lys
755 760 765

AAT ATT TTC AAA ACA TTA ATC ATA TTG TTA ACC ATA TGT GCA TTA TAC 2352
Asn Ile Phe Lys Thr Leu Ile Ile Leu Leu Thr Ile Cys Ala Leu Tyr
770 775 780

GCA TTG GTG GTT GGA TTT GTG TTT GTT ATC AAT ACT ATT GCT ACT TTT 2400
Ala Leu Val Val Gly Phe Val Phe Val Ile Asn Thr Ile Ala Thr Phe
785 790 795 800

GGA ACC GGT GGA ACA TCT ACC TAT GTG CTC GTT AGT ATT GTG GTT TCA 2448
Gly Thr Gly Gly Thr Ser Thr Tyr Val Leu Val Ser Ile Val Val Ser
805 810 815

TTG TTG TCC ACC TAT GGT CTT TAT ACG TTA ATG TCC ATT TTG TAC TTG 2496
Leu Leu Ser Thr Tyr Gly Leu Tyr Thr Leu Met Ser Ile Leu Tyr Leu
820 825 830

GAC CCA TGG CAC ATG TTG ACT TGT TCT GTA CAA TAC TTT TTG ATG ATT 2544
Asp Pro Trp His Met Leu Thr Cys Ser Val Gln Tyr Phe Leu Met Ile
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CCA TCG TAC ACT TGT ACA TTA CAA ATA TTT GCA TTT TGT AAT ACT CAC 2592
Pro Ser Tyr Thr Cys Thr Leu Gln Ile Phe Ala Phe Cys Asn Thr His
850 855 860

GAT GTC TCG TGG GGT ACA AAA GGT GAC AAC AAT CCA AAA GAA GAT TTG 2640
Asp Val Ser Trp Gly Thr Lys Gly Asp Asn Asn Pro Lys Glu Asp Leu
865 870 875 880

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Ser Asn Gln Tyr Ile Ile Glu Lys Asn Ala Ser Gly Glu Phe Glu Ala
885 890 895

GTT ATT GTT GAT ACA AAT ATC GAT GAA GAT TAC CTT GAG ACA TTA TAT 2736
Val Ile Val Asp Thr Asn Ile Asp Glu Asp Tyr Leu Glu Thr Leu Tyr
900 905 910

AAT ATC AGG TCA AAG AGA TCA AAC AAA AAA GTG GCT TTG GGC CAT TCT 2784
Asn Ile Arg Ser Lys Arg Ser Asn Lys Lys Val Ala Leu Gly His Ser
915 920 925

GAA AAG ACG CCT CTT GAT GGT GAT GAT TAT GCA AAA GAC GTT CGT ACT 2832
Glu Lys Thr Pro Leu Asp Gly Asp Asp Tyr Ala Lys Asp Val Arg Thr
930 935 940

AGA GTT GTG TTG TTT TGG ATG ATT GCA AAT TTG GTA TTT ATA ATG ACC 2880
Arg Val Val Leu Phe Trp Met Ile Ala Asn Leu Val Phe Ile Met Thr
945 950 955 960

ATG GTA CAA GTT TAC GAG CCA GGT GAT ACC GGA AGA AAC ATT TAT TTG 2928
Met Val Gln Val Tyr Glu Pro Gly Asp Thr Gly Arg Asn Ile Tyr Leu
965 970 975

GCC TTT ATT TTG TGG GCA GTG GCA GTG TTG GCT CTT GTC AGA GCT ATT 2976
Ala Phe Ile Leu Trp Ala Val Ala Val Leu Ala Leu Val Arg Ala Ile
980 985 990

GGC TCT CTT GGA TAC TTG ATA CAA ACA TAT GCA CGG TTT TTT GTG GAA 3024
Gly Ser Leu Gly Tyr Leu Ile Gln Thr Tyr Ala Arg Phe Phe Val Glu
995 1000 1005

```
TCG AAG AGT AAA TGG ATG AAA CGA GGA TAT ACC GCG CCG AGT CAC AAT    3072
Ser Lys Ser Lys Trp Met Lys Arg Gly Tyr Thr Ala Pro Ser His Asn
   1010                1015                1020

CCA TTA AAT TAG                                                    3084
Pro Leu Asn
1025
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 1027 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: both
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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1               5                   10                  15
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            20                  25                  30
Leu Ser Thr Ser Gly Ser Pro Phe His Gln Gln Gln Ser Pro Arg Gln
        35                  40                  45
Ser Pro Asn Ile Phe Ser Arg Ser Thr Ala Arg Ala Thr Ser Asp Arg
    50                  55                  60
Thr Ser Pro Arg Lys Thr Tyr Gln Pro Leu Asn Phe Asp Ser Glu Asp
65                  70                  75                  80
Glu Asp Ala Lys Glu Ser Glu Phe Met Ala Ala Thr Ser Lys Leu Asn
                85                  90                  95
Met Ser Ile Tyr Asp Asn Thr Pro Asn Leu Gln Phe Asn Lys Ser Gly
            100                 105                 110
Ala Ala Thr Pro Arg Ala Gln Phe Thr Ser Lys Glu Ser Pro Lys Arg
        115                 120                 125
Gln Lys Thr Thr Glu Val Thr Ile Asp Phe Asp Asn Asp Asp Asp Asn
    130                 135                 140
Asn His Thr Leu Glu Phe Glu Asn Gly Ser Pro Arg Arg Ser Phe Arg
145                 150                 155                 160
Ser Ser Ala Ile Ser Ser Glu Arg Phe Leu Pro Pro Pro Gln Pro Ile
                165                 170                 175
Phe Ser Arg Glu Thr Phe Ala Glu Ala Asn Ser Arg Glu Glu Glu Lys
            180                 185                 190
Ser Ala Asp Gln Glu Thr Leu Asp Glu Lys Tyr Asp Tyr Asp Ser Tyr
        195                 200                 205
Gln Lys Gly Tyr Glu Glu Val Glu Thr Leu His Ser Glu Gly Thr Ala
    210                 215                 220
Tyr Ser Gly Ser Ser Tyr Leu Ser Asp Asp Ala Ser Pro Glu Thr Thr
225                 230                 235                 240
Asp Tyr Phe Gly Ala Ser Ile Asp Gly Asn Ile Met His Asn Ile Asn
                245                 250                 255
Asn Gly Tyr Val Pro Asn Arg Glu Lys Thr Ile Thr Lys Arg Lys Val
            260                 265                 270
Arg Leu Val Gly Gly Lys Ala Gly Asn Leu Val Leu Glu Asn Pro Val
        275                 280                 285
Pro Thr Glu Leu Arg Lys Val Leu Thr Arg Thr Glu Ser Pro Phe Gly
    290                 295                 300
Glu Phe Thr Asn Met Thr Tyr Thr Ala Cys Thr Ser Gln Pro Asp Thr
305                 310                 315                 320
Phe Ser Ala Glu Gly Phe Thr Leu Arg Ala Ala Lys Tyr Gly Arg Glu
                325                 330                 335
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370 375 380
Gln Val Ile Ile Val Ala Asp Gly Arg Asn Lys Val Gln Gln Ser Val
385 390 395 400
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Pro Tyr Val Asn Asn Ser Lys Val Asn Ala His Leu Phe Glu Tyr Thr
420 425 430
Thr Gln Ile Ser Ile Asp Glu Asn Leu Lys Phe Lys Gly Asp Glu Lys
435 440 445
Asn Leu Ala Pro Val Gln Val Leu Phe Cys Leu Lys Glu Leu Asn Gln
450 455 460
Lys Lys Ile Asn Ser His Arg Trp Leu Phe Asn Ala Phe Cys Pro Val
465 470 475 480
Leu Asp Pro Asn Val Ile Val Leu Leu Asp Val Gly Thr Lys Pro Asp
485 490 495
Asn His Ala Ile Tyr Asn Leu Trp Lys Ala Phe Asp Arg Asp Ser Asn
500 505 510
Val Ala Gly Ala Ala Gly Glu Ile Lys Ala Met Lys Gly Lys Gly Trp
515 520 525
Ile Asn Leu Thr Asn Pro Leu Val Ala Ser Gln Asn Phe Glu Tyr Lys
530 535 540
Leu Ser Asn Ile Leu Asp Lys Pro Leu Glu Ser Leu Phe Gly Tyr Ile
545 550 555 560
Ser Val Leu Pro Gly Ala Leu Ser Ala Tyr Arg Tyr Ile Ala Leu Lys
565 570 575
Asn His Asp Asp Gly Thr Gly Pro Leu Ala Ser Tyr Phe Lys Gly Glu
580 585 590
Asp Leu Leu Cys Ser His Asp Lys Asp Lys Glu Asn Thr Lys Ala Asn
595 600 605
Phe Phe Glu Ala Asn Met Tyr Leu Ala Glu Asp Arg Ile Leu Cys Trp
610 615 620
Glu Leu Val Ser Lys Arg Asn Asp Asn Trp Val Leu Lys Phe Val Lys
625 630 635 640
Leu Ala Thr Gly Glu Thr Asp Val Pro Glu Thr Ile Ala Glu Phe Leu
645 650 655
Ser Gln Arg Arg Arg Trp Ile Asn Gly Ala Phe Phe Ala Ala Leu Tyr
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Ser Leu Tyr His Phe Arg Lys Ile Trp Thr Thr Asp His Ser Tyr Ala
675 680 685
Arg Lys Phe Trp Leu His Val Glu Glu Phe Ile Tyr Gln Leu Val Ser
690 695 700
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705 710 715 720
Phe Leu Thr Gly Ser Leu Val Ser Tyr Lys Ser Leu Gly Lys Lys Gly
725 730 735
Gly Phe Trp Ile Phe Thr Leu Phe Asn Tyr Leu Cys Ile Gly Val Leu
740 745 750
Thr Ser Leu Phe Ile Val Ser Ile Gly Asn Arg Pro His Ala Ser Lys
755 760 765
Asn Ile Phe Lys Thr Leu Ile Ile Leu Leu Thr Ile Cys Ala Leu Tyr
770 775 780
Ala Leu Val Val Gly Phe Val Phe Val Ile Asn Thr Ile Ala Thr Phe
785 790 795 800
Gly Thr Gly Gly Thr Ser Thr Tyr Val Leu Val Ser Ile Val Val Ser
805 810 815
Leu Leu Ser Thr Tyr Gly Leu Tyr Thr Leu Met Ser Ile Leu Tyr Leu
820 825 830

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Asp Pro Trp His Met Leu Thr Cys Ser Val Gln Tyr Phe Leu Met Ile
        835                 840                 845
Pro Ser Tyr Thr Cys Thr Leu Gln Ile Phe Ala Phe Cys Asn Thr His
    850                 855                 860
Asp Val Ser Trp Gly Thr Lys Gly Asp Asn Asn Pro Lys Glu Asp Leu
865                 870                 875                 880
Ser Asn Gln Tyr Ile Ile Glu Lys Asn Ala Ser Gly Glu Phe Glu Ala
                885                 890                 895
Val Ile Val Asp Thr Asn Ile Asp Glu Asp Tyr Leu Glu Thr Leu Tyr
            900                 905                 910
Asn Ile Arg Ser Lys Arg Ser Asn Lys Lys Val Ala Leu Gly His Ser
        915                 920                 925
Glu Lys Thr Pro Leu Asp Gly Asp Asp Tyr Ala Lys Asp Val Arg Thr
    930                 935                 940
Arg Val Val Leu Phe Trp Met Ile Ala Asn Leu Val Phe Ile Met Thr
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Met Val Gln Val Tyr Glu Pro Gly Asp Thr Gly Arg Asn Ile Tyr Leu
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            980                 985                 990
Gly Ser Leu Gly Tyr Leu Ile Gln Thr Tyr Ala Arg Phe Phe Val Glu
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   1010                1015                1020
Pro Leu Asn
025
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3084 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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ATGGTTGGTA ACTTAAAACT GYCACTCCTG CTTCTACGAT TTCTTTCGCT TAAATACCGA    180
AAGGGTGACA GCTGATCACC TAGAGGTAAA GTGGTTGTCG TTAGGGGTTC TGTTAGTGGA    240
TTATAAAAAA GGTCTTCATG ACGTTCTCGT CGTTGGAGTT TCGACTTATA CTCGTATATA    300
CTATTATGGG GCTTGAATGT TAAGTTGTTT TCGCCGCGTC GGTGTGGTTC TCGTGTTAAG    360
TGTAGCTTTC TTAGAGGCTT TTCTGTTTTT TGATGACTTC ACTGGTAACT GAAACTGTTA    420
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TTGAACCAGA ACCTCTTAGG TCAAGGTTGT CTCAACTCTT TTCACAACTG GTCTTGGCTC    900
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AAAAGACGAC TTCCCAAGTG GAATTCTCGA CGGTTTATGC CGTCTCTTTG ACTCTAACAG   1020
TAAACATATT GGTACATATT ACTCCTGCTT CAACGTAAAC GGTCTTGATA CGTACCACAC   1080
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ACCTTTTTTC AAGTTCACTA TTAACAACGT CTACCATCTT TATTTCAAGT TGTTAGGCAA   1200
GAACTTAACG AATGCCGTTG TCCGACGATA GTTCTTTTAA ACCGGTCCGG GATACAGTTG   1260
TTATCGTTTC ATTTACGGGT AAACAAAACTT ATATGGTGAG TTTATAGATA GCTACTCTTG   1320
AACTTTAAGT TTCCTCTACT TTTTTTGGAA CGTGGTCAAG TTCAGAACAA GACAAACTTT   1380
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AGACACAATG GTCCACGTAA CAGACGTATA GCTATGTAAC GGAACTTTTT GGTGCTACTA   1740
CCATGTCCCG GTAACCGAAG AATAAAGTTT CCACTTCTAA ATGAGACAAG TGTACTGTTT   1800
CTGTTTCTCT TATGGTTTCG ATTGAAAAAG CTTCGTTTAT ACATGAACCG ACTTCTGTCT   1860
TAGGAAACAA CCCTTAACCA TAGTTTTTCT TTACTGTTAA CCCAAGAATT TAAACAATTT   1920
GACCGTTGGC CACTTTGACT ACAAGGACTT TGTTAACGTC TTAAAGAAAG CGTTTCTGCT   1980
TCTACCTAAT TACCACGGAA AAAACGACGA AACATGAGGA ACATAGTGAA ATCTTTTTAT   2040
ACCTGCTGAC TGGTAAGCAT ACGATCTTTT AAAACCGATG TACAGCTTCT TAAGTAAATA   2100
GTTAACCATA GTAATAACAA AAGTAAAAAA AGAAACTCAT TAAAGATAAA TTGTAAAATA   2160
AAAAACTGTC CAAGTAACCA CAGAATGTTT TCAGAACCAT TTTTTCCACC TAAAACCTAA   2220
AAGTGTAATA AGTTAATAGA GACATAGCCA CAAAACTGTA GAAACAAGTA ACAGAGGTAA   2280
CCATTATCTG GTGTACGTAG TTTCTTATAA AAGTTTTGTA ATTAGTATAA CAATTGGTAT   2340
ACACGTAATA TGCGTAACCA CCAACCTAAA CACAAACAAT AGTTATGATA ACGATGAAAA   2400
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CTGCAAGCAT GATCTCAACA CAACAAAACC TACTAACGTT TAAACCATAA ATATTACTGG   2880
TACCATGTTC AAATGCTCGG TCCACTATGG CCTTCTTTGT AAATAAACCG GAAATAAAAC   2940
ACCCGTCACC GTCACAACCG AGAACAGTCT CGATAACCGA GAGAACCTAT GAACTATGTT   3000
TGTATACGTG CCAAAAAACA CCTTAGCTTC TCATTTACCT ACTTTGCTCC TATATGGCGC   3060
GGCTCAGTGT TAGGTAATTT AATC                                          3084
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1734 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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CAGCTTATTG TAGGTGCTCC TTTCATTATT CGTACTTCCT ACCCCATGGA GTTTAAAATG    240
ATAAYYGAAA TTTAAAGCCA ACTAGCCAAC TAGCCAACTA GCCAGCTAGC MAGMCAAGAC    300
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ATATTTATTG TAGATTACAC CCCGCTCTAC AAAGTTACCA TGAAGACAAA ACAACTTGTT    600
TGAAGTTATA TGAATCGATG TTAAAAATCT GCGTCTCGTG GAGAGTAACT TGATTATGTT    660
AGGTCTGCTA TCGTTTATAC TATGACCGCA TCATATACAG GACATTAGAG CATCCTAAAT    720
TAAATCATCC CATTGTTTCA AGTTTCTTTG TTTAGCAAAG AGACAGTTCC AACTTGTTGT    780
CGTCATAATT ATCGGAATAA TTTAAGCGAG GAAAAGTTGT GAAACAAATT GAAGAGTGGA    840
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CCACGTAATC AACAACAAGT AGCCATATAA TTCAAAATTT GTAGTAGTTG GGCAAATAAT    960
ATTTATACCC CCCCACTCCC CCAACCTTCC AATTTTCCTC TTCCTCTGGG AATTTTTTTT   1020
TTTGAAATAC AAATCTCTTT TAAAACCAAC TTAAACCTAT TAATTATGAC AATTGAATAT   1080
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GGTGATGGAA TTGGTGATAT TCCAGGGATA ATTTCTACAT TAGATTATCT TAAAAATTTA   1200
GGAATTGATA TTATTTGGTT AAGTCCAATG TATAAATCCC CTATGGAAGA TATGGGTTAT   1260
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```

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GATTGGTATA TTTGGAAACC ACCGAGAATT GACGCNAAAA ACTGGTGNAA AAATTACCAC 1500
CAAATAATTG GGGGTCATTT TTTTCAGGAT CAGCATGGGA TATGATGAAT TAACCGATGA 1560
ATATTATTTA AGATTATTTG CCAAGGGACA ACCTGATTTA AATTGGGAAA ATGAAGAAAG 1620
TCGTCAAGCA ATTTATAATT CTGCCATGAA ATCATGGTTT GATAAAGGTG TTGATGGATT 1680
TAGAATTGAT GTTGCTGGAT NATATTCTAA AGATCGACCT CNGAATCAAA GGAA 1734

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGAGGAGTCG ACATGACAGT CAACAC 26

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGCATTAAAG CTCTAGAAGA ACCACC 26

What is claimed is:

1. Substantially pure chitin synthase (CHS1) polypeptide.

2. The CHS1 of claim 1, characterized in that it has a molecular weight of about 116kD as determined by reducing SDS-PAGE.

3. The CHS1 of claim 1, having the amino acid sequence of SEQ ID NO:2 (Figure 1b-g).

4. An isolated polynucleotide encoding the CHS1 polypeptide of claim 1.

5. The polynucleotide of claim 4, having the sequence of SEQ ID NO:1 (Figure 1b-g).

6. The CHS1 of claim 1, wherein the CHS1 is derived from a yeast cell.

7. An expression vector comprising the polynucleotide of claim 4.

8. A host cell comprising the vector of claim 7.

9. An antibody that binds specifically to the CHS1 polypeptide of claim 1.

10. A method for inhibiting the growth of yeast comprising contacting the yeast with an inhibiting effective amount of a reagent which suppresses CHS1 activity.

11. The method of claim 10, wherein the reagent is a CHS1 antisense sequence.

12. The method of claim 10, wherein the yeast is *Candida albicans*.

13. The method of claim 10, wherein the reagent is an anti-CHS1 antibody.

14. A method for determining whether a compound affects CHS1 activity, said method comprising:

a) incubating the compound with CHS1 polypeptide, or with a recombinant cell expressing CHS1 under conditions sufficient to allow the components to interact; and

b) determining the effect of the compound on CHS1 activity or expression.

15. The method of claim 14, wherein the effect is inhibition of CHS1 activity

16. A vector for identifying a eukaryotic regulatory polynucleotide which is capable of regulating gene expression in a prokaryotic host cell, comprising:

a) a selectable marker gene;

b) at the 5' terminus of the marker gene, a restriction site at which a eukaryotic regulatory polynucleotide can be inserted to regulate expression of said marker gene; and

c) a polynucleotide which facilitates integration of the vector into the genome of said prokaryotic cell.

17. The vector of claim 16, wherein the marker gene is an auxotrophic gene.

18. The vector of claim 17, wherein the auxotrophic gene is URA3.

19. A host cell *E. coli* comprising the vector of claim 16.

20. A method for identifying a eukaryotic regulatory polynucleotide, said method comprising

a) providing a vector comprising

(i) a selectable marker gene;

(ii) at the 5′ terminus of the marker gene, a restriction endonuclease site at which a eukaryotic regulatory polynucleotide can be inserted to regulate expression of said marker gene; and

(iii) a polynucleotide which facilitates integration of the vector into the genome of a predetermined cell;

b) inserting genomic DNA of a eukaryotic organism into said vector at said restriction site;

c) inserting the resultant eukaryotic polynucleotide-containing vector into a host cell;

d) detecting the selectable marker as an indication that the inserted eukaryotic polynucleotide is a regulatory polynucleotide.

21. The method of claim 20, wherein the eukaryote is a fungal pathogen.

22. The method of claim 21, wherein the fungal pathogen is selected from the group consisting of *Candida albicans, Rhodotorula sp., Saccharomyces cerevisiae, Blastoschizomyces capitatus, Histoplasma capsulatum, Aspergillus fumigatus, Coccidioides immitis, Paracoccidioides brasiliensis, Blastomyces dermatitidis,* and *Cryptococcus neoformans.*

23. The method of claim 20, wherein the marker gene is an auxotrophic gene.

24. The method of claim 23, wherein the auxotrophic gene is URA3.

25. The method of claim 20, wherein the predetermined cell is eukaryotic.

26. The method of claim 20, wherein the predetermined cell is prokaryotic.

27. A library of host cells, wherein each host cell contains a vector according to claim 16.

28. An isolated regulatory polynucleotide characterized in that it is induced by maltose and repressed by glucose.

29. The polynucleotide of claim 28 having the sequence of SEQ ID NO:4 (Figure 3a-b).

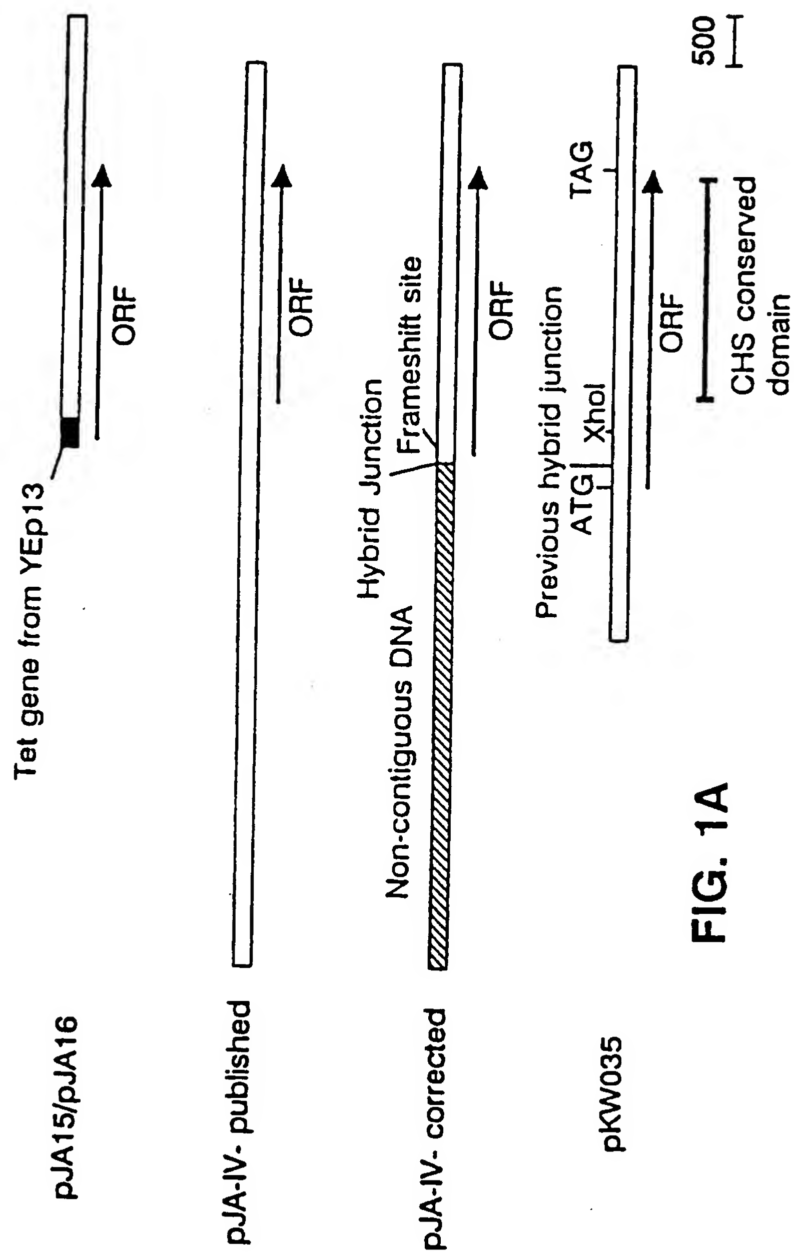
30. The polynucleotide of claim 28, wherein the polynucleotide is derived from a yeast cell.

31. A method for determining whether a polynucleotide encodes a growth-associated polypeptide, said method comprising:

a) incubating a cell comprising the polynucleotide operably linked with the regulatory polynucleotide of claim 28, under conditions which repress the regulatory polynucleotide; and

b) determining the effect on the growth of the cell.

32. The method of claim 31, wherein the effect is inhibition of cell growth.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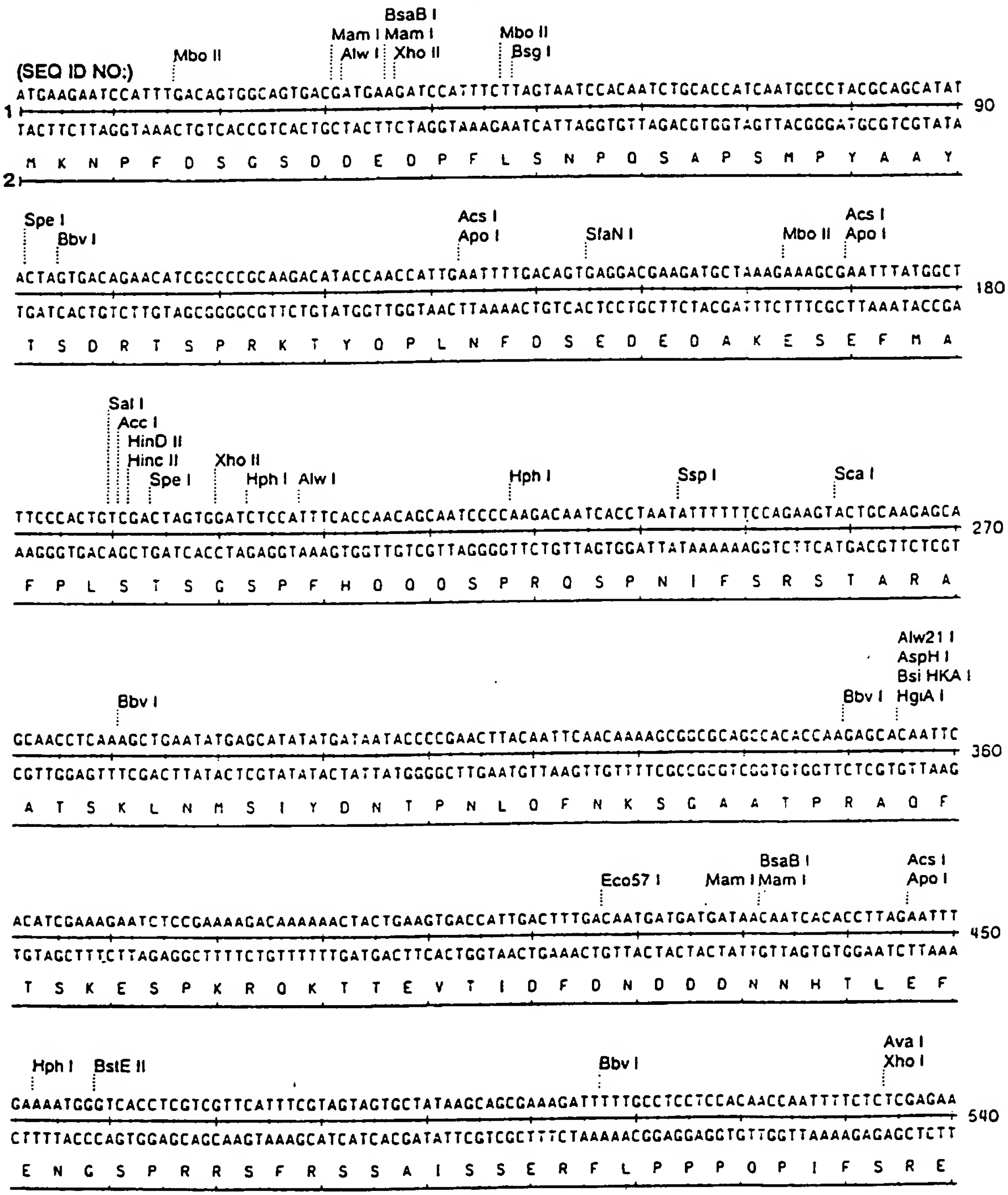
Tet gene from YEp13
ORF
pJA-IV- published
ORF
pJA-IV- corrected
Non-contiguous DNA
Hybrid Junction
Frameshift site
ORF
pKW035
Previous hybrid junction
ATG
XhoI
TAG
ORF
500
CHS conserved
domain

FIG. 1A

2/14


(SEQ ID NO:)
Mbo II
Mam I
Alw I
BsaB I
Mam I
Xho II
Mbo II
Bsg I
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1
90
TACTTCTTAGGTAAACTGTCACCGTCACTGCTACTTCTAGGTAAAGAATCATTAGGTGTTAGACGTGGTAGTTACGGGATGCGTCGTATA
M K N P F D S G S D D E D P F L S N P Q S A P S M P Y A A Y
2
Spe I
Bbv I
Acs I
Apo I
SfaN I
Mbo II
Acs I
Apo I
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TGATCACTGTCTTGTAGCGGGGCGTTCTGTATGGTTGGTAACTTAAAACTGTCACTCCTGCTTCTACGATTTCTTTCGCTTAAATACCGA
T S D R T S P R K T Y Q P L N F D S E D E D A K E S E F M A
Sal I
Acc I
HinD II
Hinc II
Spe I
Xho II
Hph I
Alw I
Hph I
Ssp I
Sca I
TTCCCACTGTCGACTAGTGGATCTCCATTTCACCAACAGCAATCCCCAAGACAATCACCTAATATTTTTTCCAGAAGTACTGCAAGAGCA
270
AAGGGTGACAGCTGATCACCTAGAGGTAAAGTGGTTGTCGTTAGGGGTTCTGTTAGTGGATTATAAAAAAGGTCTTCATGACGTTCTCGT
F P L S T S G S P F H Q Q Q S P R Q S P N I F S R S T A R A
Bbv I
Bbv I
Alw21 I
AspH I
Bsi HKA I
HgiA I
GCAACCTCAAAGCTGAATATGAGCATATATGATAATACCCCGAACTTACAATTCAACAAAAGCGGCGCAGCCACACCAAGAGCACAATTC
360
CGTTGGAGTTTCGACTTATACTCGTATATACTATTATGGGGCTTGAATGTTAAGTTGTTTTCGCCGCGTCGGTGTGGTTCTCGTGTTAAG
A T S K L N M S I Y D N T P N L Q F N K S G A A T P R A Q F
Eco57 I
Mam I
BsaB I
Mam I
Acs I
Apo I
ACATCGAAAGAATCTCCGAAAAGACAAAAAACTACTGAAGTGACCATTGACTTTGACAATGATGATGATAACAATCACACCTTAGAATTT
450
TGTAGCTTTCTTAGAGGCTTTTCTGTTTTTTGATGACTTCACTGGTAACTGAAACTGTTACTACTACTATTGTTAGTGTGGAATCTTAAA
T S K E S P K R Q K T T E V T I D F D N D D D N N H T L E F
Hph I
BstE II
Bbv I
Ava I
Xho I
GAAAATGGGTCACCTCGTCGTTCATTTCGTAGTAGTGCTATAAGCAGCGAAAGATTTTTGCCTCCTCCACAACCAATTTTCTCTCGAGAA
540
CTTTTACCCAGTGGAGCAGCAAGTAAAGCATCATCACGATATTCGTCGCTTTCTAAAAACGGAGGAGGTGTTGGTTAAAAGAGAGCTCTT
E N G S P R R S F R S S A I S S E R F L P P P Q P I F S R E

FIG. 1B

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```
Eco57 I, Mbo II, Mbo II
ACATTTGCTGAAGCCAACTCCCGTGAAGAAGAAAAATCGGCAGATCAAGAAACATTAGATGAAAAATACGATTATGATTCATACCAGAAG 630
TGTAAACGACTTCGGTTGAGGGCACTTCTTCTTTTTAGCCGTCTAGTTCTTTGTAATCTACTTTTTATGCTAATACTAAGTATGGTCTTC
 T  F  A  E  A  N  S  R  E  E  E  K  S  A  D  Q  E  T  L  D  E  K  Y  D  Y  D  S  Y  Q  K

BsrD I, BsaM I, Bsm I, SfaN I, BseN I, Bsr I, Fok I, Sfc I
GGTTATGAGGAAGTAGAAACATTGCATTCGGAAGGTACAGCTTATAGTGGCTCATCTTATTTGTCGGATGATGCCAGTCCTGAAACTACA 720
CCAATACTCCTTCATCTTTGTAACGTAAGCCTTCCATGTCGAATATCACCGAGTAGAATAAACAGCCTACTACGGTCAGGACTTTGATGT
 G  Y  E  E  V  E  T  L  H  S  E  G  T  A  Y  S  G  S  S  Y  L  S  D  D  A  S  P  E  T  T

Mun I, Ssp I, BsaA I, BsaA I, SnaB I
GATTACTTTGGAGCTTCAATTGATGGTAATATTATGCACAACATTAACAATGGATACGTACCAAATAGAGAAAAAACCATTACCAAAAGA 810
CTAATGAAACCTCGAAGTTAACTACCATTATAATACGTGTTGTAATTGTTACCTATGCATGGTTTATCTCTTTTTTGGTAATGGTTTTCT
 D  Y  F  G  A  S  I  D  G  N  I  M  H  N  I  N  N  G  Y  V  P  N  R  E  K  T  I  T  K  R

BspM I, BseN I, Bsr I, HinD II, Hinc II
AAAGTGAGATTAGTTGGTGGCAAAGCAGGTAACTTGGTCTTGGAGAATCCAGTTCCAACAGAGTTGAGAAAAGTGTTGACCAGAACCGAG 900
TTTCACTCTAATCAACCACCGTTTCGTCCATTGAACCAGAACCTCTTAGGTCAAGGTTGTCTCAACTCTTTTCACAACTGGTCTTGGCTC
 K  V  R  L  V  G  G  K  A  G  N  L  V  L  E  N  P  V  P  T  E  L  R  K  V  L  T  R  T  E

Alw26 I, BsmA I, Ple I, Hph I, Dra III, ApaL I, Alw21 I, AspH I, Bsi HKA I, HgiA I, Bbv I, Hph I, Bbv I, Afl II, Eco57 I
TCTCCATTTGGTGAGTTTACCAACATGACATACACAGCGTGCACTTCGCAGCCAGATACTTTTTCTGCTGAAGGGTTCACCTTAAGAGCT 990
AGAGGTAAACCACTCAAATGGTTGTACTGTATGTGTCGCACGTGAAGCGTCGGTCTATGAAAAAGACGACTTCCCAAGTGGAATTCTCGA
 S  P  F  G  E  F  T  N  M  T  Y  T  A  C  T  S  Q  P  D  T  F  S  A  E  G  F  T  L  R  A

Ppu10 I, Nsi I
GCCAAATACGGCAGAGAAACTGAGATTGTCATTTGTATAACCATGTATAATGAGGACGAAGTTGCATTTGCCAGAACTATGCATGGTGTG 1080
CGGTTTATGCCGTCTCTTTGACTCTAACAGTAAACATATTGGTACATATTACTCCTGCTTCAACGTAAACGGTCTTGATACGTACCACAC
 A  K  Y  G  R  E  T  E  I  V  I  C  I  T  M  Y  N  E  D  E  V  A  F  A  R  T  M  H  G  V
```

FIG. 1C

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Alw21 I, AspH I, Bsi HKA I, HgiA I; AccB7 I, PfiM I, PfiM I, Van91 I

```
ATGAAAAATATCGCTCATTTGTGCTCACGCCATAAATCCAAAATATGGGGCAAAGATAGCTGGAAAAAAGTTCAAGTGATAATTGTTGCA 1170
TACTTTTTATAGCGAGTAAACACGAGTGCGGTATTTAGGTTTTATACCCCGTTTCTATCGACCTTTTTTCAAGTTCACTATTAACAACGT
 M  K  N  I  A  H  L  C  S  R  H  K  S  K  I  W  G  K  D  S  W  K  K  V  Q  V  I  I  V  A
```

Bbv I; Acs I, Apo I; Clr I, Eae I; Bal I, MluNI, Msc I, Msc I; Dra II, Eco 0109 I, EcoO109 I; HinD II, Hinc II

```
GATGGTAGAAATAAAGTTCAACAATCCGTTCTTGAATTGCTTACGGCAACAGGCTGCTATCAAGAAAATTTGGCCAGGCCCTATGTCAAC 1260
CTACCATCTTTATTTCAAGTTGTTAGGCAAGAACTTAACGAATGCCGTTGTCCGACGATAGTTCTTTTAAACCGGTCCGGGATACAGTTG
 D  G  R  N  K  V  Q  Q  S  V  L  E  L  L  T  A  T  G  C  Y  Q  E  N  L  A  R  P  Y  V  N
```

Cla I; Acs I, Apo I

```
AATAGCAAAGTAAATGCCCATTTGTTTGAATATACCACTCAAATATCTATCGATGAGAACTTGAAATTCAAAGGAGATGAAAAAAACCTT 1350
TTATCGTTTCATTTACGGGTAAACAAACTTATATGGTGAGTTTATAGATAGCTACTCTTGAACTTTAAGTTTCCTCTACTTTTTTTGGAA
 N  S  K  V  N  A  H  L  F  E  Y  T  T  Q  I  S  I  D  E  N  L  K  F  K  G  D  E  K  N  L
```

BseN I, Bsr I

```
GCACCAGTTCAAGTCTTGTTCTGTTTGAAAGAACTGAACCAAAAGAAAATCAATTCCCATAGATGGCTTTTTAATGCCTTTTGTCCTGTC 1440
CGTGGTCAAGTTCAGAACAAGACAAACTTTCTTGACTTGGTTTTCTTTTAGTTAAGGGTATCTACCGAAAAATTACGGAAAACAGGACAG
 A  P  V  Q  V  L  F  C  L  K  E  L  N  Q  K  K  I  N  S  H  R  W  L  F  N  A  F  C  P  V
```

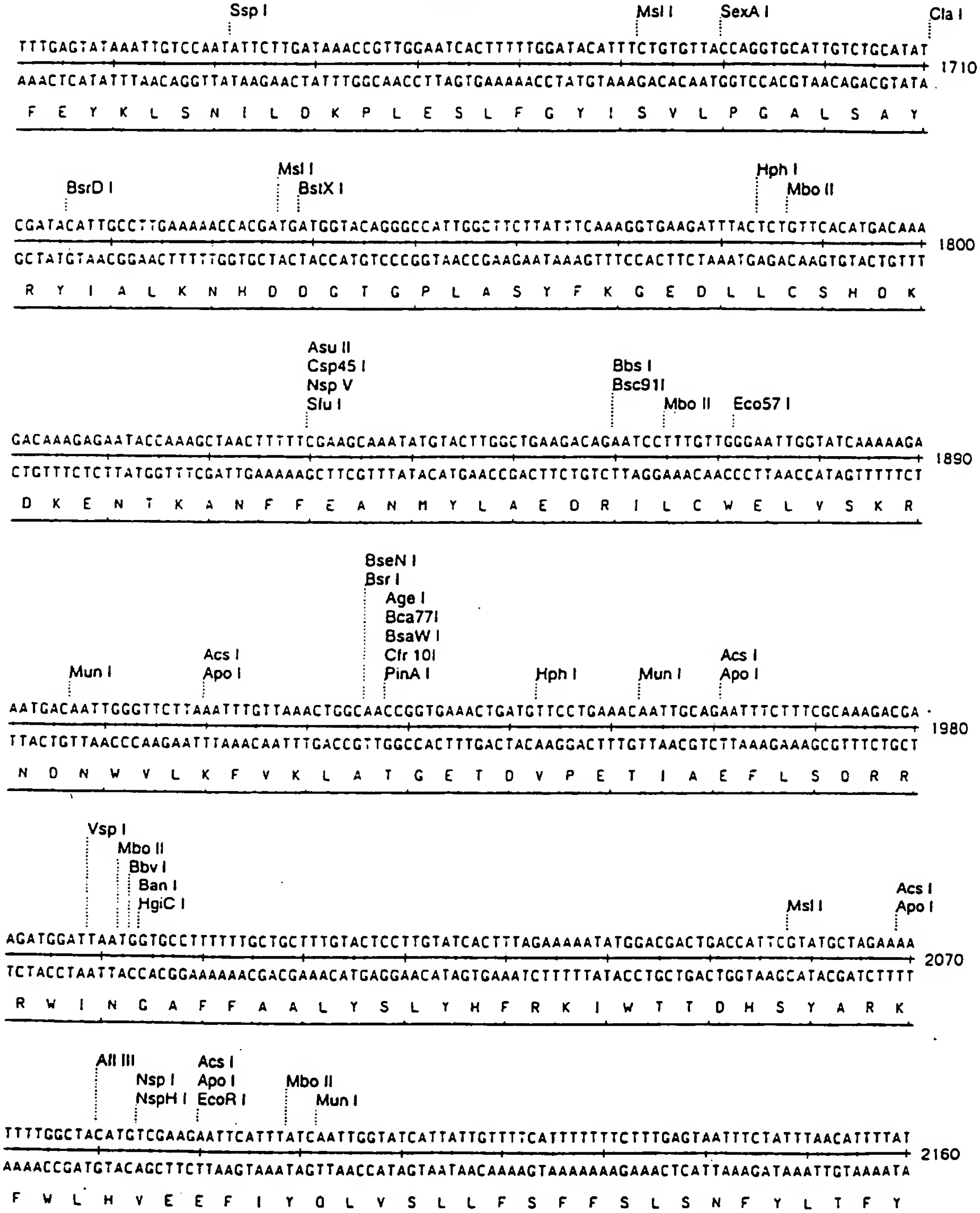
Acc65 I, Asp 718, Ban I, Bcg I', HgiC I; Kpn I; Bcg I; Asp 700, BsaM I, Bsm I, Xmn I

```
TTGGACCCCAATGTTATTGTTCTTTTAGATGTGGGTACCAAACCCGATAACCATGCCATTTATAATCTATGGAAAGCATTCGATAGAGAT 1530
AACCTGGGGTTACAATAACAAGAAAATCTACACCCATGGTTTGGGCTATTGGTACGGTAAATATTAGATACCTTTCGTAAGCTATCTCTA
 L  D  P  N  V  I  V  L  L  D  V  G  T  K  P  D  N  H  A  I  Y  N  L  W  K  A  F  D  R  D
```

Bbv I; Alwn I; Hph I; Vsp I; Hga I; Acs I, Apo I

```
TCCAATGTAGCAGGGGCTGCTGGTGAAATTAAAGCGATGAAAGGTAAAGGTTGGATTAATCTTACAAATCCATTAGTTGCGTCACAGAAT 1620
AGGTTACATCGTCCCCGACGACCACTTTAATTTCGCTACTTTCCATTTCCAACCTAATTAGAATGTTTAGGTAATCAACGCAGTGTCTTA
 S  N  V  A  G  A  A  G  E  I  K  A  M  K  G  K  G  W  I  N  L  T  N  P  L  V  A  S  Q  N
```

FIG. 1D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Msl I
SexA I
Cla I
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1710
AAACTCATATTTAACAGGTTATAAGAACTATTTGGCAACCTTAGTGAAAAACCTATGTAAAGACACAATGGTCCACGTAACAGACGTATA
F E Y K L S N I L D K P L E S L F G Y I S V L P G A L S A Y
BsrD I
Msl I
BstX I
Hph I
Mbo II
CGATACATTGCCTTGAAAAACCACGATGATGGTACAGGGCCATTGGCTTCTTATTTCAAAGGTGAAGATTTACTCTGTTCACATGACAAA
1800
GCTATGTAACGGAACTTTTTGGTGCTACTACCATGTCCCGGTAACCGAAGAATAAAGTTTCCACTTCTAAATGAGACAAGTGTACTGTTT
R Y I A L K N H D D G T G P L A S Y F K G E D L L C S H D K
Asu II
Csp45 I
Nsp V
Sfu I
Bbs I
Bsc91I
Mbo II
Eco57 I
GACAAAGAGAATACCAAAGCTAACTTTTTCGAAGCAAATATGTACTTGGCTGAAGACAGAATCCTTTGTTGGGAATTGGTATCAAAAAGA
1890
CTGTTTCTCTTATGGTTTCGATTGAAAAAGCTTCGTTTATACATGAACCGACTTCTGTCTTAGGAAACAACCCTTAACCATAGTTTTTCT
D K E N T K A N F F E A N M Y L A E D R I L C W E L V S K R
BseN I
Bsr I
Age I
Bca77I
BsaW I
Cfr 10I
PinA I
Acs I
Apo I
Mun I
Hph I
Mun I
Acs I
Apo I
AATGACAATTGGGTTCTTAAATTTGTTAAACTGGCAACCGGTGAAACTGATGTTCCTGAAACAATTGCAGAATTTCTTTCGCAAAGACGA
1980
TTACTGTTAACCCAAGAATTTAAACAATTTGACCGTTGGCCACTTTGACTACAAGGACTTTGTTAACGTCTTAAAGAAAGCGTTTCTGCT
N D N W V L K F V K L A T G E T D V P E T I A E F L S D R R
Vsp I
Mbo II
Bbv I
Ban I
HgiC I
Acs I
Apo I
Msl I
AGATGGATTAATGGTGCCTTTTTTGCTGCTTTGTACTCCTTGTATCACTTTAGAAAAATATGGACGACTGACCATTCGTATGCTAGAAAA
2070
TCTACCTAATTACCACGGAAAAAACGACGAAACATGAGGAACATAGTGAAATCTTTTTATACCTGCTGACTGGTAAGCATACGATCTTTT
R W I N G A F F A A L Y S L Y H F R K I W T T D H S Y A R K
All III
Nsp I
NspH I
Acs I
Apo I
EcoR I
Mbo II
Mun I
TTTTGGCTACATGTCGAAGAATTCATTTATCAATTGGTATCATTATTGTTTTCATTTTTTTCTTTGAGTAATTTCTATTTAACATTTTAT
2160
AAAACCGATGTACAGCTTCTTAAGTAAATAGTTAACCATAGTAATAACAAAAGTAAAAAAAGAAACTCATTAAAGATAAATTGTAAAATA
F W L H V E E F I Y Q L V S L L F S F F S L S N F Y L T F Y

FIG. 1E

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TTTTTGACAGGTTCATTGGTGTCTTACAAAAGTCTTGGTAAAAAAGGTGGATTTTGGATTTTCACATTATTCAATTATCTCTGTATCGGT 2250
AAAAACTGTCCAAGTAACCACAGAATGTTTTCAGAACCATTTTTTCCACCTAAAACCTAAAAGTGTAATAAGTTAATAGAGACATAGCCA
F L T G S L V S Y K S L G K K G G F W I F T L F N Y L C I G

Alw26 I
BsmA I
Ppu10 I
Nsp I
NspH I
Nsi I
SfaN I
Ssp I
Asp 700
Xmn I
Vsp I
HinD II
Hinc II
Hpa I
Nde I

GTTTTGACATCTTTGTTCATTGTCTCCATTGGTAATAGACCACATGCATCAAAGAATATTTTCAAAACATTAATCATATTGTTAACCATA 2340
CAAAACTGTAGAAACAAGTAACAGAGGTAACCATTATCTGGTGTACGTAGTTTCTTATAAAAGTTTTGTAATTAGTATAACAATTGGTAT
V L T S L F I V S I G N R P H A S K N I F K T L I I L L T I

Age I
Bca77I
BsaW I
Cfr 10I
PinA I
Alw21 I
AspH I
Bsi HKA I
HgiA I

TGTGCATTATACGCATTGGTGGTTGGATTTGTGTTTGTTATCAATACTATTGCTACTTTTGGAACCGGTGGAACATCTACCTATGTGCTC 2430
ACACGTAATATGCGTAACCACCAACCTAAACACAAACAATAGTTATGATAACGATGAAAACCTTGGCCACCTTGTAGATGGATACACGAG
C A L Y A L V V G F V F V I N T I A T F G T G G T S T Y V L

Xcm I
Dsa I
Nco I
Sty I
Afl III
Nsp I
NspH I
HinD II
Hinc II

GTTAGTATTGTGGTTTCATTGTTGTCCACCTATGGTCTTTATACGTTAATGTCCATTTTGTACTTGGACCCATGGCACATGTTGACTTGT 2520
CAATCATAACACCAAAGTAACAACAGGTGGATACCAGAAATATGCAATTACAGGTAAAACATGAACCTGGGTACCGTGTACAACTGAACA
V S I V V S L L S T Y G L Y T L M S I L Y L D P W H M L T C

Bsp1407 I
BsrG I
Bsp1407 I
BsrG I
Ssp I
Alw26 I
BsmA I

TCTGTACAATACTTTTTGATGATTCCATCGTACACTTGTACATTACAAATATTTGCATTTTGTAATACTCACGATGTCTCGTGGGGTACA 2610
AGACATGTTATGAAAAACTACTAAGGTAGCATGTGAACATGTAATGTTTATAAACGTAAAACATTATGAGTGCTACAGAGCACCCCATGT
S V Q Y F L M I P S Y T C T L Q I F A F C N T H D V S W G T

Hph I
Mbo II
BseN I
Bsr I
Acs I
Apo I

AAAGGTGACAACAATCCAAAAGAAGATTTGAGTAATCAGTACATTATTGAGAAAAATGCCAGTGGAGAATTTGAGGCTGTTATTGTTGAT 2700
TTTCCACTGTTGTTAGGTTTTCTTCTAAACTCATTAGTCATGTAATAACTCTTTTTACGGTCACCTCTTAAACTCCGACAATAACAACTA
K G D N N P K E D L S N Q Y ! I E K N A S G E F E A V I V D

Cla I
Alw26 I
Mbo II
BsmA I

ACAAATATCGATGAAGATTACCTTGAGACATTATATAATATCAGGTCAAAGAGATCAAACAAAAAAGTGGCTTTGGGCCATTCTGAAAAG 2790
TGTTTATAGCTACTTCTAATGGAACTCTGTAATATATTATAGTCCAGTTTCTCTAGTTTGTTTTTTCACCGAAACCCGGTAAGACTTTTC
T N I D E D Y L E T L Y N I R S K R S N K K V A L G H S E K

FIG. 1F

Acy I
Aha II
BsaH I
Hin1 I
Hga I
Hph I
Acs I
Apo I
Fok I
Dsa I
Nco I
Sly I

ACGCCTCTTGATGGTGATGATTATGCAAAAGACGTTCGTACTAGAGTTGTGTTGTTTTGGATGATTGCAAATTTGGTATTTATAATGACC
TGCGGAGAACTACCACTACTAATACGTTTTCTGCAAGCATGATCTCAACACAACAAAACCTACTAACGTTTAAACCATAAATATTACTGG 2880

T P L D G D D Y A K D V R T R V V L F W M I A N L V F I M T

Bca77I
BsaW I
Hph I
Mbo II

ATGGTACAAGTTTACGAGCCAGGTGATACCGGAAGAAACATTTATTTGGCCTTTATTTTGTGGGCAGTGGCAGTGTTGGCTCTTGTCAGA
TACCATGTTCAAATGCTCGGTCCACTATGGCCTTCTTTGTAAATAAACCGGAAATAAAACACCCGTCACCGTCACAACCGAGAACAGTCT 2970

M V Q V Y E P G D T G R N I Y L A F I L W A V A V L A L V R

Nde I
Eam1104 I
Ear I
Ksp632 I
Mbo II
Fok I
KspI

GCTATTGGCTCTCTTGGATACTTGATACAAACATATGCACGGTTTTTTGTGGAATCGAAGAGTAAATGGATGAAACGAGGATATACCGCG
CGATAACCGAGAGAACCTATGAACTATGTTTGTATACGTGCCAAAAAACACCTTAGCTTCTCATTTACCTACTTTGCTCCTATATGGCGC 3060

A I G S L G Y L I Q T Y A R F F V E S K S K W M K R G Y T A

Ple I

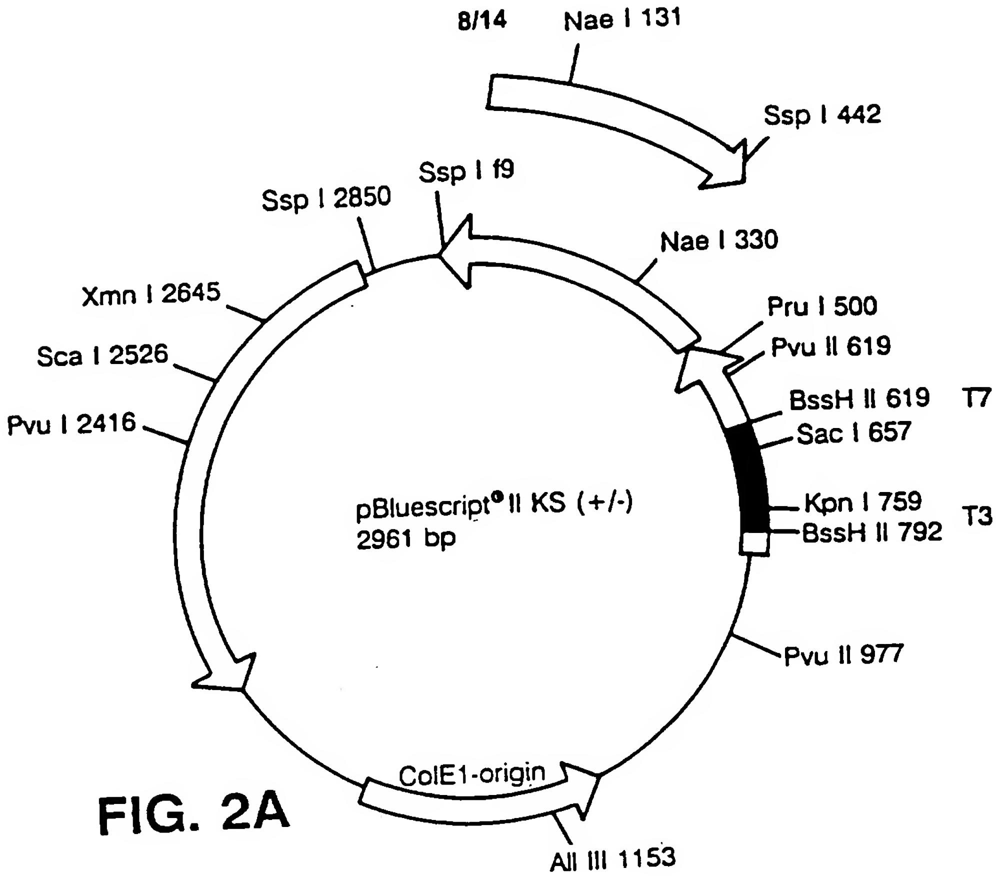
CCGAGTCACAATCCATTAAATTAG
GGCTCAGTGTTAGGTAATTTAATC 3084

P S H N P L N .

(SEQ ID NO: 1)
(SEQ ID NO: 3)

(SEQ ID NO: 2)

FIG. 1G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Pru I 500
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Sca I 2526
BssH II 619
T7
Pvu I 2416
Sac I 657
pBluescript® II KS (+/-)
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Kpn I 759
T3
BssH II 792
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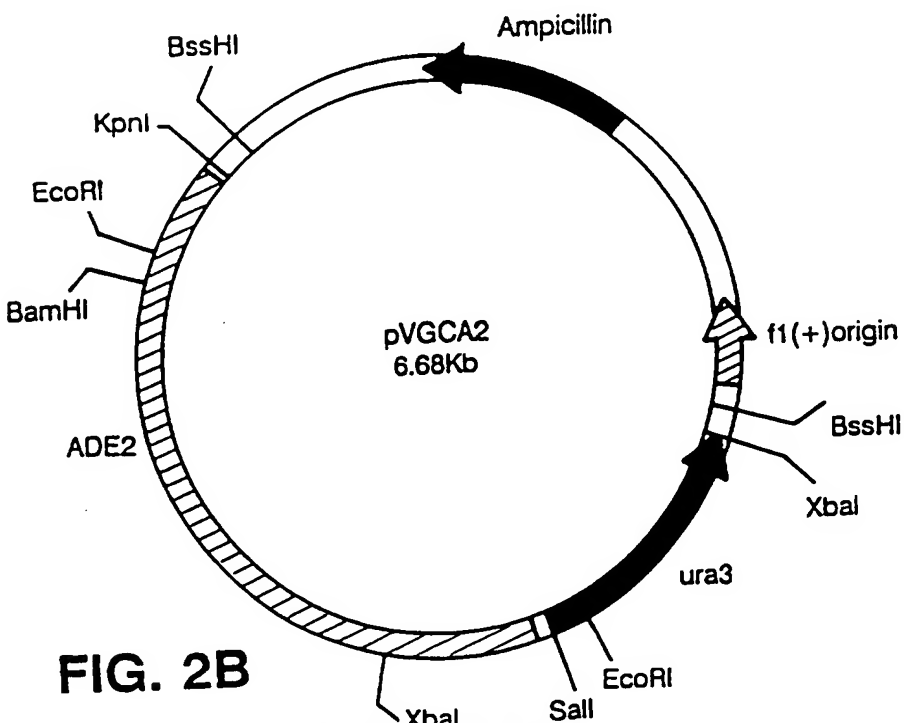
Afl III 1153

FIG. 2A


Ampicillin
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KpnI
EcoRI
BamHI
pVGCA2
6.68Kb
f1(+)origin
BssHI
ADE2
XbaI
ura3
EcoRI
SalI
XbaI

FIG. 2B

(SEQ ID NO: 4)

```
         10         20         30         40
ATAATCGTTG TGCTACTGGT AGCTAGtTTC TGCTCTCTCA 40
CTATAxGGTC tTAGTGTTGA CTGTCATGTC GATCAAGTTA 80
CTTACAGGTA AATTATTGAG TTTCAATAAG GTTGGTTTCG 120
TTGTGGCTAG TTTTTTCGAT GTTTTACAAA ATGAAAAAAA 160
ACTTAATACA TTTAAGCCAA CAGCTTATTG TAGGTGCTCC 200
        210        220        230        240
TTTCATTATT CGTACTTCCT ACCCCATGGA GTTTAAAATG 240
ATAAYYGAAA TTTAAAGCCA ACTAGCCAAC TAGCCAACTA 280
GCCAGCtagC MAGMCAAgAC AAAACTAATC ACAAAGACTA 320
AAAGAAAGTG TAGTTATAAA TCATTGCGAG AATTATTGCG 360
AAAxGATATT CCGCTTTTCA AAAAAACATT ATTGCGAAAA 400
        410        420        430        440
TCATTGCxGA xGAAAGGGGG AGTTATTTTT GGGGTACTAC 440
TATGCATGTG TTGTTGTCAA TGTCTACCAC AAAAAGGGGC 480
TTCTTTCAAT TGATAAACCT ACCAAAACAT CTGGTAATCA 520
AAAGCTACTT GTGTGAGACT ATATTTATTG TAGATTACAC 560
CCCGCTCTAC AAAGTTACCA TGAAGACAAA ACAACTTGTT 600
        610        620        630        640
TGAAGTTATA TGAATCGATG TTAAAaATCT GCGTCTCGTG 640
GAGAGTAACT TGATTATGTT AGGTCTGCTA TCGTTTATAC 680
TATGACCGCA TCATATACAG GACATTAGAG CATCCTAAAT 720
TAAATCATCC CATTGTTTCA AGTTTCTTTG TTTAGCAAAG 760
AGACAGTTCC AACTTGTTGT CGTCATAATT ATCGGAATAA 800
```

FIG. 3A

810 820 830 840

TTTAAGCGAG GAAAAGTTGT GAAACAAATT GAAGAGTGGA 840
GTGTGGGGGA GGGGGAGGGA AACAAGGAAG TATACCTCCA 880
CCAAGTAGAA CCCAAATACT CCACGTAATC AACAACAAGT 920
AGCCATATAA TTCAAAATTT GTAGTAGTTg GGCAAATAAT 960
ATTTATACCC CCCCACTCCC CCAACCTTCC AATTTTCCTC 1000

1010 1020 1030 1040

TTCCTCTGGG AATTTTTTTT TTTGAAATAC AAATCTCTTT 1040
TAAAACCAAC TTAAACCTAT TAATTATGAC AATTGAATAT 1080
ACTTGGTGGA AAGACGCTAC TATTTATCAA ATTTGGCCTG 1120
CTTCATATAA AGATTCCAAT GGTGATGGAA TTGGTGATAT 1160
TCCAGGGATA ATTTCTACAT TAGATTATCT TAAAAATTTA 1200

1210 1220 1230 1240

GGAATTGATA TTATTTGGTT AAGTCCAATG TATAAATCCC 1240
CTATGGAAGA TATGGGTTAT GATATTAGTG ATTATGAATC 1280
TATAAATCCT GATTTTGGTA CTATGGAAGA CATGCAAAAT 1320
TTAATTGATG GATGTCATGA AAGAGGAATG AAAATTATTT 1360
GTGATTTAGT AGTTAATCAT ACATCATCTG AACATGAATG 1400

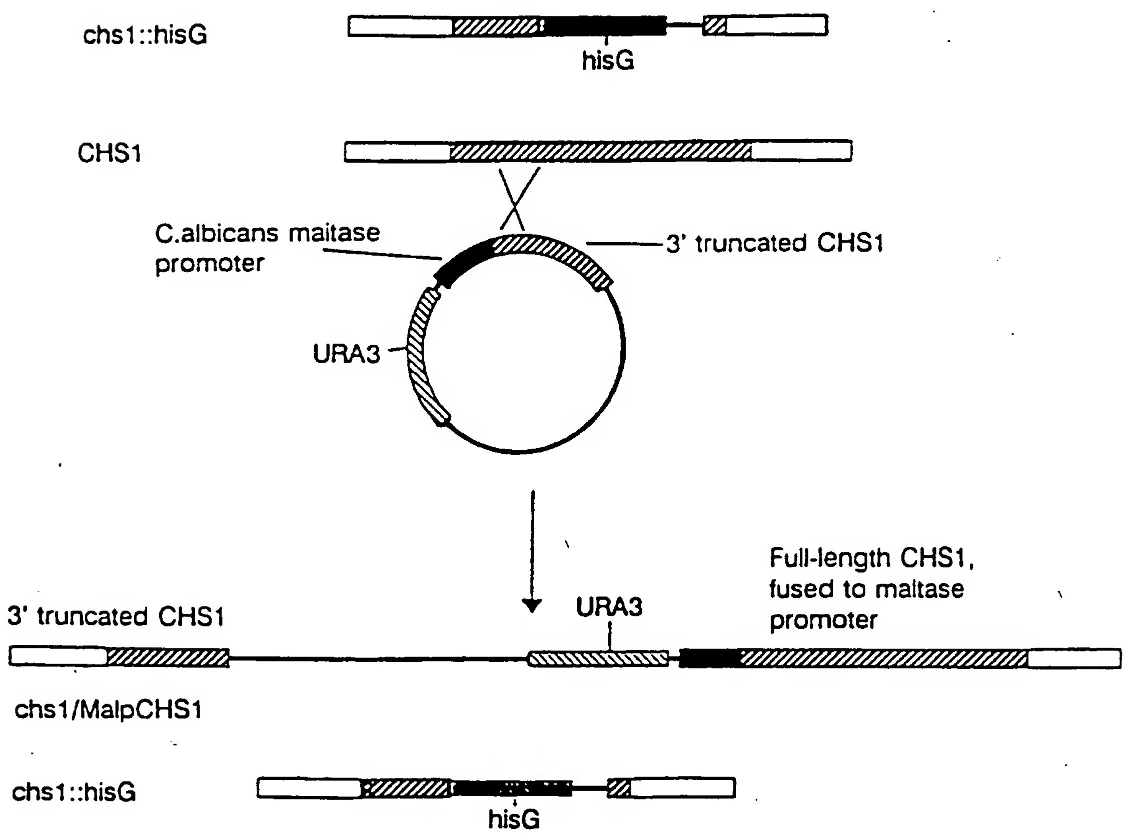
1410 1420 1430 1440

GTTTAAACAA TCAAGATCAC TGAAATCAAA CCCTAAAAGA 1440
GATTGGTATA TTTGGAAACC ACCGAGAATT GACGCxAAAA 1480
ACTGGTGxAA AAATTACCAC CAAATAATTG GGGGTCATTT 1520
TTTTCAGGAT CAGCATGGGA TATGATGAAT TAACCGATGA 1560
aTATTATTTA AGaTTATTTG CCAAGGGACA ACCTGATTTA 1600

1610 1620 1630 1640

AATTGGGAAA ATGAAGAAAG TCGTCAAGCA ATTTATAATT 1640
CTGCCATGAA ATCATGGTTT GATAAAGGTG TTGATGGATT 1680
TAGAATTGAT GTTGCTGGAT XATATTCTAA AGATCGACCT 1720
CxGAATCAAA GGAA 1734

FIG. 3B



Transform with maltose as carbon source,
switch to glucose to repress expression of CHS1 ·HS1

FIG. 4

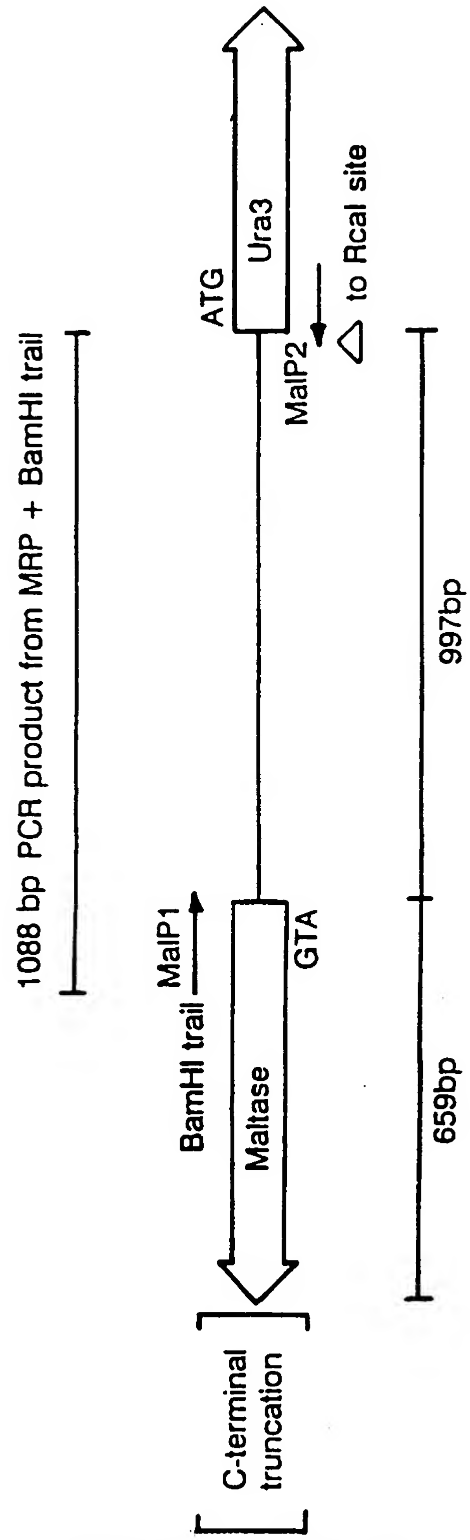

1088 bp PCR product from MRP + BamHI trail
ATG
Ura3
to RcaI site
MalP2
997bp
MalP1
BamHI trail
GTA
Maltase
659bp
C-terminal truncation

FIG. 5

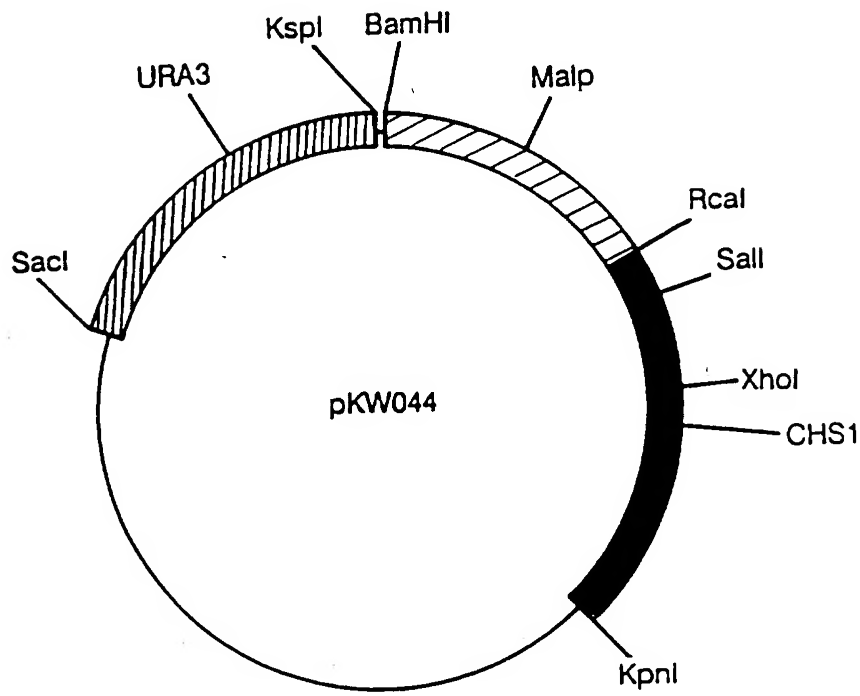


FIG. 6

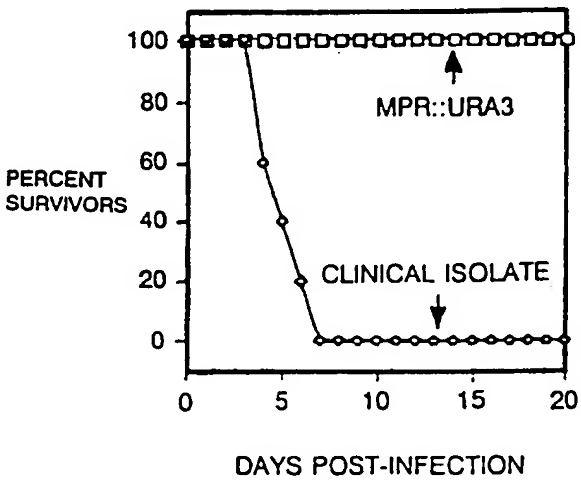


FIG. 7A

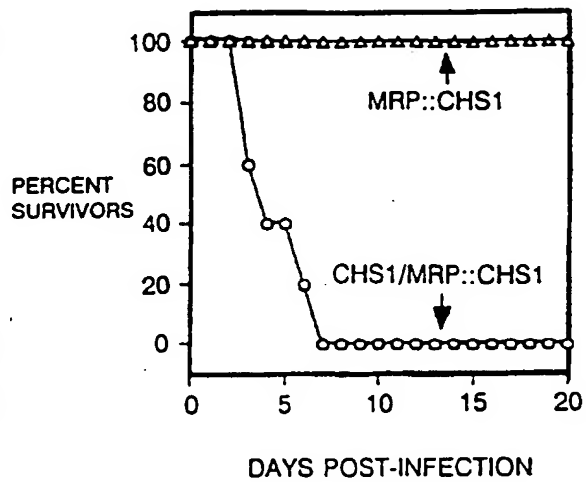


FIG. 7B

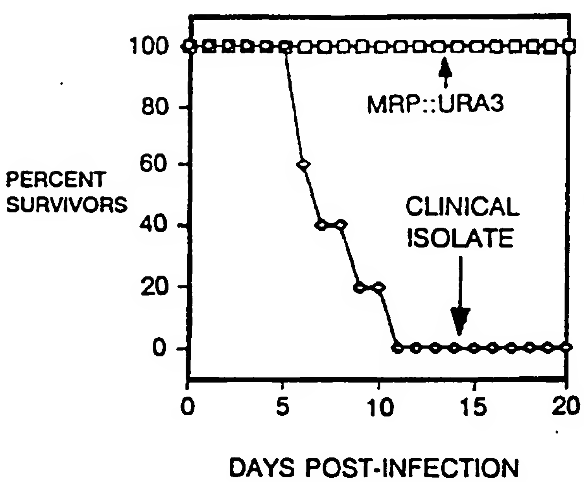


FIG. 7C

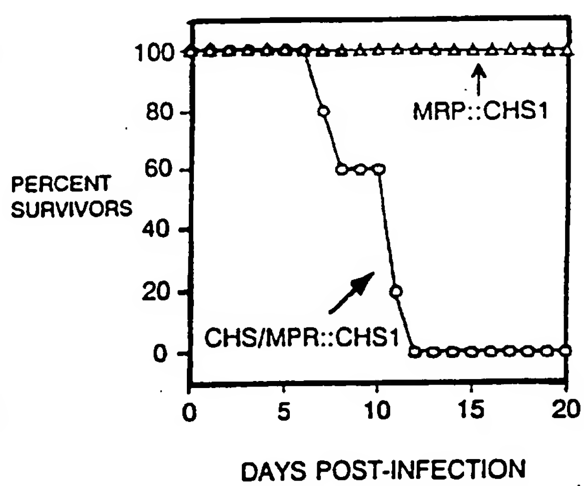


FIG. 7D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17459

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 9/10, 1/15, 1/21; C07K 16/14, 16/40; C07H 21/04
US CL :435/193, 240.1, 243; 530/387.1; 536/23.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/193, 240.1, 243; 530/387.1; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	AU-YOUNG et al. Isolation of a chitin synthase gene (CSH1) from Candida albicans by expression in Saccharomyces cerevisiae. Molecular Microbiology. February 1990, Vol. 4, No. 2, pages 197-207, especially p. 199 and Figures 3 and 6.	1-8 ----- 9,14,15
Y	CHOI et al. The use of divalent cations and pH for the determination of specific yeast chitin synthases. Analytical Biochemistry. June 1994, Vol. 219, pages 368-372, especially Figure 3.	14,15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 FEBRUARY 1997	28 FEB 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	BRIAN LATHROP
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17459

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	SEMINO et al. Homologs of the Xenopus developmental gene DG42 are present in zebrafish and mouse and are involved in the synthesis of Nod-like chitin oligosaccharides during early embryogenesis. Proceedings of the National Academy of Sciences of the Unitied States of America. 14 May 1996, Vol. 93, No. 10, pages 4548-4553, the entire article.	9
X,P ----- Y,P	SUDOH, M. Candida albicans CACHS1A gene for chitin synthase I, complete cds. Direct submission to GenBank: Accession No. D43627. 10 April 1996.	4-5 ---- 1-3, 6-9, 14,15
Y	Database Medline on STN, US National Library of Medicine (Bethesda, MD, USA), No. 92378414, VALDES et al. 'Antigens specific to pre-cysts and in vivo chitin synthetase activity in Entamoeba invadens,' abstract, Archivos de Investigacion Medica, 1990, Vol. 21, Supplement 1, page 223.	9
A	BULAWA, C.E. Genetics and molecular biology of chitin synthesis in fungi. Annual Review of Microbiology. 1993, Vol. 47, pages 505-534, especially pages 525-526.	1-8, 14,15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17459

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 14-15

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17459

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

EMBL, GENBANK, EST-STS, PIR, SWISS-PROT, A-GENESEQ, USPAT, MEDLINE, WPIDS REGISTRY
search terms: SEQ ID NOS:1 and 2, chitin (2w) synth?, csh1, antibodies, assay

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-9 and 14-15, drawn to chitin synthase polypeptide, DNA encoding the same, an expression vector and host cell comprising the DNA encoding the protein, an antibody directed against the protein, and a method of using the protein to screen for interacting compounds.
Group II, claims 10-12, drawn to a method of using antisense polynucleotides.
Group III, claims 10, 12, and 13, drawn to method of using the antibody raised against the protein.
Group IV, claims 16-19 and 27, drawn to a vector and host cells comprising the same.
Group V, claims 20-26, drawn to a method of using said vector to identify eukaryotic regulatory polynucleotides.
Group VI, claims 28-30, drawn to an isolated regulatory polynucleotide.
Group VII, claims 31-32, drawn to a method of using said isolated regulatory polynucleotide.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of Group I is the chitin synthase polypeptide. Groups II and III are drawn to methods of using distinct products, an antisense polynucleotide and an antibody. Groups IV-VII are drawn to distinct products and methods of using these products. The products of Group IV-VII do not require chitin synthase or the encoding DNA and hence are not related by the same special technical feature.